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STUDIES IN THE PHYSIOLOGY OF THE FUNGI

VI. The Relation of Bacteria to Cellulose Fermentation Induced by Fungi, with Special Reference to the Decay of Wood

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It is a matter of common observation and easy demonstration that bacteria are invariably present when wood decays under ordinary conditions. That a majority of these forms would be the ordinary air and soil saprophytes is only to be expected, yet the presence of cellulose-dissolving forms is not precluded. The purpose of this investigation is to determine whether or not cellulose-dissolving bacteria are habitually present on decaying wood under natural conditions and their influence, if any, on the rate of decay, also to determine whether or not the ordinary saprophytic forms, so universally present, in any way affect the cellulose-dissolving proclivities of the fungus actually causing the decay, by changing the reaction of the substratum so that it may be more or less favorable for optimum growth.

Due to the facts that all wood used in this work was first sterilized by autoclaving and that the theories regarding the effect of autoclaving wood are somewhat diverse, it was found desirable, if not expedient, to determine first whether or not this process of sterilizing wood produced new conditions, so that the results would not be comparable to those which might obtain under natural conditions.

SURVEY OF LITERATURE

EFFECTS OF AUTOCLAVING WOOD

The different viewpoints in regard to the effects of sterilization upon woody tissues are indicated by the works of Potter ('04) and Spaulding ('06). The earlier workers in this field were primarily interested in the determination of the chemical compounds present in woody tissues. Thus, Singer ('82) extracted four distinct substances by means of boiling water: vanillin, a substance giving the reactions of coniferin, a water-soluble gum, and also a substance giving a yellow color with hydrochloric acid. Van Wisselingh ('98) found that after treating slices of *Beta vulgaris* with distilled water at 125° C. for six hours a wall of pure cellulose remained, while the pectin substances were decomposed and dissolved. Even at a temperature of 100° C. the tendency for water to decompose and dissolve the cell wall was noted.

Potter ('04) tried the effect of hot water on different woods, *Quercus, Ulmus, Alnus, Aesculus,* and others, and found that steaming had a decided effect on the cell wall. It was shown that the action of boiling water caused a delignification of the xylem. The watery extract obtained from sawdust and fragments of wood when treated in this manner contained a substance which reacts to the lignin tests. Still further, it was shown that cold water, operating for a longer period of time, had a similar power of extracting from the xylem a substance which reacts with phloroglucin and thallin sulphate, and thus by continued soaking in water wood undergoes a partial delignification. If this be true, then autoclaving, even for the time required for sterilization, may have a marked effect on the wood when used for experimental purposes with reference to decay induced by fungi.

Rose and Lisse ('17) have made chemical analyses of sound Douglas fir and of the same wood in various stages of decay. Their results showed a rapid decrease in the percentage of cellulose as decay proceeded, while the lignin seemed to be far more resistant. They do not state what fungus caused the decay of the wood upon which they worked, and for that

reason their results are far less valuable and can in no way be taken as general. It has been shown by von Schrenk ('00) that even on the same kind of wood two fungi may act in an entirely different manner with respect to which portion of the woody tissue is utilized. Thus, *Polyporus juniperinus* growing on the wood of *Juniperus virginiana* caused a delignification in the first stages of decay and the remaining wood fiber was completely reduced to cellulose. *Polyporus carneus,* on the other hand, almost entirely removed the cellulose when growing on the same wood.

Spaulding ('06) differs to some extent from the views expressed by Potter. Steaming tests were made with a variety of woods, thin sections being placed in the Arnold sterilizer or the autoclave and the effects noted by micro-chemical reactions. Spaulding obtained slight delignification in *Sassafras Sassafras* after fifteen hours of boiling, and in *Picea rubens* after seven hours in the autoclave or with interrupted boiling after a total of twenty-seven hours. With these two exceptions forty hours of boiling seemed to have no effect upon the lignin of the sections. In other tests, using the autoclave at a temperature of about 120° C., it was found that many woods gave evidences of delignification after fifteen hours of treatment, and nearly all after eighteen hours.

Spaulding, however, does not consider these effects important with reference to experimental work with the wood-decaying fungi, since it is claimed even the very thin sections had to be subjected to the action of boiling water for relatively long intervals before any decided effect could be detected. In discussing the effects of boiling water on finely divided wood, however, he admits that lignin is extracted in sufficient quantity for lignin reactions to be obtained in the filtrate from the extracted material. The effect of autoclaving thin shavings for one hour was also tested by Zeller ('17). It was found that the sections were not delignified to such an extent that by staining with zinc chloriodid any change could be detected, although the water in which the shavings had been boiled gave a very faint pink color with phloroglucin and hydrochloric acid.

THE RELATION OF BACTERIA TO CELLULOSE FERMENTATION

The fact that certain microörganisms have the power to dissolve cellulose has been known for some time. The actual determination of the forms involved and a better understanding of the process resulted from more recent investigations.

Mitscherlich ('50) first noted that the cellulose in the cell walls of slices of potato was dissolved when the slices were immersed in water and kept in a warm place for several days. A few years later attention was called by Haubner to the fact that it was impossible to recover more than 50 per cent of the crude fiber fed to ruminants. This observation was soon confirmed by numerous other workers, but the agent causing this reduction was unknown at that time. It has been recently demonstrated by Ellenberger ('15) and others that cellulose-dissolving ferments could not be extracted from the intestines of herbivorous animals, nor could any such ferments be obtained from the walls of the stomach or any of the secretive glands. The fungous flora of the intestines, however, showed numerous cellulose-dissolving organisms.

Some ten years after Haubner's discovery Trécul ('65) described a new genus, *Amylobacter,* which he found in connection with macerated plant tissues. Following this work numerous other workers described what they thought to be new forms. Thus we find *Bacterium navicula* of Reinke and Berthold ('79), the hydrogen and methane ferments of Omelianski, *Clostridium polymyxa, Vibrio rugula, Clostridium butyricum* of Prazmowski ('80), and others, all having the power to dissolve cellulose. At first there was considerable confusion regarding the validity of these forms. Van Tieghem ('77), working on Trécul's *Amylobacter*, found it to be a motile rod and called it *Bacillus amylobacter.* Prazmowski ('80) regarded his *Clostridium butyricum* as synonymous with *Vibrion butyrique* Pasteur, *Amylobacter* Trécul, *Bacillus amylobacter* Van Tieghem, and *Bacterium navicula* Reinke and Berthold. The work of Omelianski ('95, '97, '02, '04) greatly contributed to a clearer conception of the causative organisms in cellulose fermentation and a better understanding of the processes involved.

It is of special interest to note that *Bacillus amylobacter*, as shown by Van Tieghem ('77), does not dissolve the cellulose from all plants, and also the fact, as indicated by Omelianski, that with the forms worked on by him the power to dissolve cellulose decreased as the age of the cultures increased. McBeth and Scales ('13) found that cellulose-dissolving bacteria are universally present in cultivated soils.

That even the ordinary saprophytes may, under some conditions, affect some of the components of the cell wall, is shown by van Hall ('02). He found that *Bacillus subtilis* and *B. vulgatus* could produce a rot on potatoes, turnips, and various nuts at a temperature of from 37 to 42° C. and that the middle lamella was dissolved. Potter ('04) also found that in the decoction made by boiling the alburnum of oak wood *Bacillus subtilis* could grow and utilize the extracted substance (lignin).

Experimental Work

The effects of autoclaving wood

Color changes induced in Douglas fir and western hemlock.—Equal amounts, 2.000 gms., of fine Douglas fir sawdust were placed in 125-cc. Erlenmeyer flasks and 25 cc. of distilled water were added to each. The flasks and contents were then weighed to the closest 0.1 gram preparatory to autoclaving, so that if any change in weight took place during autoclaving the flask could be restored to its original weight by the addition of distilled water, any change in the intensity of color then not being due to an increased concentration of the contents. The same methods were followed with western hemlock sawdust.

Some flasks and contents were not autoclaved; others were autoclaved for increasing intervals from forty-five minutes to twenty-one hours and the change of color noted. The colors are those of Ridgway ('12).

In both cases it is evident that a decided darkening occurs as the length of autoclaving increases. For the purposes of sterilization twenty hours of autoclaving are not necessary, yet there is a marked change in color caused by forty-five minutes of this treatment. The exact nature of these changes

TABLE I

COLOR CHANGES INDUCED BY AUTOCLAVING DOUGLAS FIR AND WESTERN HEMLOCK SAWDUST FOR VARIOUS INTERVALS

Interval of autoclaving	Color	
	Douglas fir	Western hemlock
Control (not autoclaved)	Antimony-yellow	Cinnamon-buff
45 minutes	Cinnamon-buff	Sayal-brown
10 hours	Cinnamon	Snuff-brown
20 hours	Mikado-brown	Natural brown

is not entirely known but it is quite evident that some changes must occur due to the prolonged heating.

Relative amounts of reducing substances in the aqueous extracts after autoclaving Douglas fir sawdust.—Into each of fifteen thoroughly cleaned 125-cc. Erlenmeyer flasks equal amounts, 2.000 gms., of fine Douglas fir sawdust were placed. The sawdust was prepared from the heart-wood in the following manner: Large pieces were sawed in the direction of the grain, resulting in a rather fine to coarse sawdust. This was then ground in a bone grinder until a comparatively fine mixture was obtained. The sawdust was dried to constant weight in a hot-air oven at a temperature of 85° C. and then allowed to stand uncovered in the laboratory for twenty-four hours. It was assumed that a fairly constant weight would be attained during that time, the amount of moisture taken up depending on the relative humidity of the air and the temperature. This procedure was followed rather than that of taking equal amounts of thoroughly dry sawdust, since it was found that a considerable fluctuation in weight took place during the process of weighing due to absorption of atmospheric moisture.

To each of the flasks 25 cc. of distilled water were added, and each flask and contents weighed, as before, to the nearest 0.1 gm., in order that the flasks might be restored to their original weight after autoclaving. Three of the fifteen flasks were not autoclaved, three were autoclaved 45 minutes, three 1 hour and 45 minutes, three 11 hours, and three 21 hours. After removal of the last set of flasks from the autoclave the contents of all the flasks were emptied into graduate cylinders and the total contents brought up to 80 cc. The extracts were then

filtered through No. 4 Whatman filter-paper and the filtrate tested for reducing substances by Shaffer's ('14) method for the determination of sugars.

In table II the amounts of reducing substances, calculated as mgs. of copper, are given. Ten cc. of the sample were used in each case. The potassium permanganate solution used in the titration was 1/20 normal. The reduction value for the Fehling control has been subtracted.

TABLE II

AMOUNT OF REDUCING SUBSTANCES CALCULATED AS MILLIGRAMS OF COPPER IN 10 CC. OF THE AQUEOUS EXTRACTS

Flask no.	Interval of autoclaving	Amount of reducing substances in 10 cc. of the extracts	Average
45	Not autoclaved	2.22	
46		2.22	
47		2.22	2.22
1	45 minutes	5.88	
2		6.04	
3		5.72	5.88
4	1 hour, 45 minutes	7.68	
5		7.52	
7		7.68	7.63
8	11 hours	10.99	
9		11.13	
10		.....	11.06
11	21 hours	20.98	
12		21.08	
13		18.76	20.27

It is clearly evident that the amount of reducing substances present in the aqueous extracts gradually increases with an increase in the time of autoclaving. It is quite probable also that some of the reducing power of the aqueous extracts is due to the presence of sugars. Fromherz ('07) has shown that on treating wood with hot water under pressure considerable quantities of sugar may be formed from the lignocelluloses and other wood components. It is claimed by Schwalbe ('11) that as pure a cellulose as cotton will yield considerable quantities of sugar when subjected to the same treatment.

Reducing substances in the extracts of Douglas fir after autoclaving and subsequent treatment with 0.5 per cent sulphuric acid.—The purpose of this series is to determine whether or not previous autoclaving in any way affects the rate of acid hydrolysis of woody tissues.

Flasks containing Douglas fir sawdust and distilled water were prepared and autoclaved as before. Enough c.p. sulphuric acid was then added to each flask to make it 0.5 per cent acid, and the entire series again autoclaved for 1 hour at 15 pounds pressure and the amounts of reducing substances determined as mgs. of copper.

TABLE III

AMOUNT OF REDUCING SUBSTANCES CALCULATED AS MILLIGRAMS OF COPPER IN 10 CC. OF THE EXTRACTS

Flask no.	Interval of autoclaving with dist. H_2O	Amount of reducing substance in 10 cc. of the extracts	Average
40 41	Not autoclaved	55.97 55.97	55.97
14 15	45 minutes	72.22 57.08	64.65
17 19	11 hours	58.83 58.83	58.83
23 24	21 hours	61.05 63.60	62.32

The results here show but a slight increase, if any, in the amounts of reducing substances present in the wood extracts in the flasks regardless of the increased time of autoclaving with distilled water. This may indicate either that prolonged previous treatment with distilled water did not cause any changes in the wood which would make it more subject to acid hydrolysis or that 0.5 per cent sulphuric acid is of sufficient concentration to hydrolyze the wood in any condition.

Reducing substances in the extracts of Douglas fir after autoclaving and subsequent treatment with 1.0 per cent sodium hydroxide.—Flasks containing 2.000 gms. of Douglas fir sawdust were prepared in exactly the same manner as in the two

previous series. After autoclaving the flasks and contents for varying intervals with distilled water enough c. p. sodium hydroxide was added to each flask to make the contents 1.0 per cent alkaline, and the entire series was again autoclaved for one hour at fifteen pounds and the amount of reducing substances determined as mgs. of copper.

TABLE IV

AMOUNTS OF REDUCING SUBSTANCES CALCULATED AS MILLIGRAMS OF COPPER IN 10 CC. OF THE EXTRACTS

Flask no.	Interval of autoclaving with dist. H_2O	Amount of reducing substances in 10 cc. of the extracts	Average
43 44	Not autoclaved	6.99 7.63	7.31
25 27	45 minutes	4.08 4.40	4.24
28 29	11 hours	5.72 6.36	6.04
33 36	21 hours	9.54 9.38	9.46

Omitting those flasks which had no previous autoclaving there is a steady increase in the amount of reducing substances as the length of previous autoclaving increases. In this series different substances from those affected by acids would, of course, be acted upon. One to two per cent solutions of alkalis, while having but little effect upon cellulose at temperatures considerably above 100° C., break down the pectic bodies and hydrolyze gums and resins which are but slightly affected with dilute acids.

Total amount of soluble substances present in the extracts.—Five cc. of the various wood extracts were placed in carefully cleaned, dry watch glasses. The liquid was evaporated in a hot-air oven at a temperature of 90° C., the heating being continued until the weight of the residue remained constant. The following table shows the total dry weight in 5 cc. of the ex-

tracts when the sawdust was autoclaved with distilled water for the time indicated:

TABLE V

TOTAL DRY WEIGHT OF THE SOLUBLE SUBSTANCES IN THE AQUEOUS EXTRACTS

Flask no.	Interval of autoclaving	Dry wt. of residue (gms.)	Average (gms.)
45	Not autoclaved	0.0045	
46		0.0045	
47		0.0047	0.0046
1	45 minutes	0.0078	
2		0.0072	
3		0.0077	0.0076
4	1 hour, 45 minutes	0.0076	
5		0.0075	
7		0.0075	0.0075
8	11 hours	0.0114	
9		0.0113	
10		0.0112	0.0113
11	21 hours	0.0149	
12		0.0137	
13		0.0143	0.0143

A considerable increase in the total soluble substances is evident. This is no doubt due in part to the dissolving out of initially soluble substances, but that it may also be partly due to the conversion of some of the wood substances is indi-

TABLE VI

TOTAL DRY WEIGHT OF THE SOLUBLE SUBSTANCES IN THE ACID EXTRACTS

Flask no.	Interval of autoclaving with dist. H_2O	Subsequent autoclaving after addition of acid	Dry wt. of residue (gms.)	Average (gms.)
39	Not autoclaved	1 hour	0.0169	
40			0.0173	
41			0.0199	0.0180
14	45 minutes	1 hour	0.0073	
15			0.0045	
16			0.0079	0.0066
17	11 hours	1 hour	0.0088	
19			0.0080	
21			0.0075	0.0081
22	21 hours	1 hour	0.0177	
23			0.0193	
24			.....	0.0185

cated by the table showing a similar increase in the amounts of reducing substances present.

The same procedure was followed with the extracts containing 0.5 per cent sulphuric acid. Table VI shows the dry weight of the total soluble substances after the weight of sulphuric acid had been deducted in each case. Each individual watch glass was weighed immediately after taking out of the oven, to avoid the error which might be caused by the acid taking up atmospheric moisture.

Table VII shows the total dry weight of the soluble substances present in the alkaline extracts after the amount of sodium hydroxide in the residue had been deducted in each case.

TABLE VII

TOTAL DRY WEIGHT OF THE SOLUBLE SUBSTANCES IN THE ALKALINE EXTRACTS

Flask no.	Interval of autoclaving with dist. H_2O	Subsequent autoclaving after addition of alkali	Dry wt. of residue (gms.)	Average (gms.)
42	Not autoclaved	1 hour	0.0366	
43			0.0312	
44			0.0333	0.0337
25	45 minutes	1 hour	0.0384	
26			0.0385	
27			0.0377	0.0382
28*	11 hours	1 hour	0.0100	
29*			0.0029	
31			.....	0.0064
32	21 hours	1 hour	0.0383	
33			0.0415	
36			0.0413	0.0404

* Portion of extract lost in oven.

Here again a slight increase is noted as the interval of autoclaving with distilled water increased.

Relative amounts of tannins present in the aqueous extracts.—It could legitimately be contended that the increase in the amounts of reducing substances present in the extracts which reduce Fehling's solution might merely be due to prolonged boiling extracting greater amounts of tannin. With this in mind the aqueous extracts were treated with a solution of ferric chloride and the intensity of the color taken as a crite-

rion of the relative amounts of tannins present. Five cc. of the various aqueous extracts were placed in clean test-tubes of 18 mm. diameter, four drops of a 2.0 per cent aqueous solution of ferric chloride added to each, and the color determined by comparing them with Ridgway's ('12) plates.

TABLE VIII

RELATIVE AMOUNTS OF TANNINS PRESENT IN THE AQUEOUS EXTRACTS

Flask no.	Interval of autoclaving	Color	Order of intensity
45, 46, 47	Not autoclaved	Wood-brown	3
1, 2, 3	45 minutes	Yew-green	1
4, 5, 7	1 hour, 45 minutes	Krönberg's green	2
8, 9, 10	11 hours	Wood-brown	3
11, 12, 13	21 hours	Wood-brown	3
Control (distilled water)		Yellow tint	

This would seem to indicate that the extracts from the wood autoclaved the longest intervals and the extract from the wood not autoclaved contained the least tannin, while that autoclaved for forty-five minutes contained the most. Comparing these results with the table showing the amounts of reducing substances in the various aqueous extracts it is found that they in no way coincide.

The presence of lignin in the aqueous extracts.—A test for the presence of lignin in the various aqueous extracts was made with phloroglucin and hydrochloric acid. In no case was more than a faint pink color evident. When, however, a few cc. of ether were added, the mixture well shaken, the ether separated and evaporated, the residue gave a decided red color with phloroglucin and hydrochloric acid. All of the extracts gave a positive test when treated in this manner.

The presence of coniferin in the aqueous extracts.—When an aqueous solution of phenol and hydrochloric acid was added to the extracts in no case did a color reaction take place.

Hydrogen ion concentration of the aqueous extracts.—The hydrogen ion concentration of the extracts was determined according to the method suggested by Sörensen ('09) as modified by Henderson ('09) and previously employed by Duggar

in this laboratory. The following table gives the results of these determinations:

TABLE IX

HYDROGEN ION CONCENTRATION OF THE AQUEOUS EXTRACTS

Flask no.	Interval of autoclaving	Hydrogen ion concentration
45, 46, 47	Not autoclaved	10^{-7}
1, 2, 3	45 minutes	10^{-6}
4, 5, 7	1 hour, 45 minutes	10^{-6}
8, 9, 10	11 hours	10^{-5}
11, 12, 13	21 hours	10^{-4}

The extract from the unautoclaved Douglas fir sawdust is practically neutral. With the autoclaved sawdust there is a decided increase in the hydrogen ion concentration in the aqueous extracts as the interval of autoclaving increases.

Acidity of the aqueous extracts.—The extracts were also titrated against N/20 sodium hydroxide with the following results. The acidity is expressed according to Fuller's scale, each being the average of three titrations.

TABLE X

DEGREE OF ACIDITY OF THE AQUEOUS EXTRACTS

Flask no.	Interval of autoclaving	Acidity
46, 47	Not autoclaved	1.0
2, 3	45 minutes	1.0
5, 7	1 hour, 45 minutes	2.0
8, 9	11 hours	3.0
12, 13	21 hours	4.0

These results correspond with the hydrogen ion determinations. A gradual increase in acidity results from increased length of autoclaving.

Effect of autoclaving on the rate of decay induced by fungi.—In order to determine whether or not autoclaving in any way affected the rate of decay induced by fungi approximately equal amounts of western hemlock sawdust were placed in 125-cc. wide-necked bottles. The bottles and contents were placed in the hot-air oven and dried to constant weight at a temperature of 90° C. and then weighed. Approximately equal

amounts of distilled water were added to each bottle and they were then autoclaved for periods from 45 minutes to 20 hours.

The bottles in which the contents were not autoclaved were treated as follows: After drying to constant weight in the hot-air oven they were plugged with previously sterilized plugs. Weighing was done with the plugs removed, every precaution being taken to avoid contamination. Sterile distilled water was then added and they were ready for inoculation. Portions of the contents of three test bottles treated in this manner were poured on sterile agar plates and in each case they were found to be sterile.

All bottles were inoculated with *Fomes pinicola* and the rate of decay determined by the loss in weight of the sawdust at the end of four months. The inoculations were made on April 16, 1917. On August 18, 1917, the plugs were removed and the contents again dried to constant weight and the loss in weight determined. Each series contained eight cultures.

The details of this work are discussed under the relation of bacteria to the rate of decay induced by fungi.

Table XI clearly shows that autoclaving western hemlock has a marked effect on the rate of decay induced by *Fomes pinicola*. It is quite probable that for different fungi the effect might be quite different. In this case a gradual increase in the rate of decay is noted with an increase in time of autoclaving up to ten hours. After twenty hours of autoclaving the fungus did not attack the wood even though each bottle, in this case, was inoculated the second time. There was no growth of the fungus from the immediate point of inoculation, and when, at the time of the second inoculation, 30 days after the first, the small mass of fungus and agar used for the first inoculation was removed from the bottles and transferred to agar slants the fungus was found to be dead. At some point between ten and twenty hours' autoclaving a decided change in the sawdust must have taken place which made it toxic to the fungus. Just what this change was is not known. Suffice it here to say that when rate of decay induced by fungi is studied the time of autoclaving of the wood must also receive due consideration.

TABLE XI

THE EFFECT OF AUTOCLAVING WESTERN HEMLOCK ON THE RATE OF DECAY INDUCED BY FOMES PINICOLA

I	II	III	IV	V	VI	VII	VIII
Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight (gms.)	Loss (per cent)	Average loss (per cent)	Time of autoclaving	Remarks
434	7.34	7.33	0.01	0.13		Not autoclaved	Only slight growth of fungus near point of inoculation.
655	7.16	7.16	0.00	0.00			
242	7.58	7.58	0.00	0.00			
160	7.25	7.25	0.00	0.00			
106	6.96	6.96	0.00	0.00			
582	7.21	7.21	0.00	0.00	0.01		
16	7.19	7.18	0.01	0.14		45 minutes	Light growth on surface.
558	7.90	7.89	0.01	0.13			
741	7.71	7.71	0.00	0.00			
595	8.94	8.92	0.02	0.22			
244	7.46	7.44	0.02	0.27	0.15		
724	8.09	8.07	0.02	0.23		5 hours	Light growth throughout entire mass of sawdust.
598	7.00	6.96	0.04	0.57			
99	6.86	6.82	0.04	0.58			
209	7.49	7.42	0.07	0.93			
120	7.47	7.42	0.05	0.67			
762	7.67	7.62	0.05	0.65			
652	6.88	6.87	0.01	0.14	0.54		
739	6.95	6.35	0.60	8.63		10 hours	Heavy growth throughout entire mass of sawdust.
744	6.64	6.48	0.16	2.41			
647	7.50	7.32	0.18	2.40			
261	6.81	6.74	0.07	1.03			
602	7.59	7.57	0.02	0.26			
477	7.30	7.26	0.04	0.55			
98	7.40	7.34	0.06	0.81	2.30		
683	7.13	7.13	0.00	0.00		20 hours	All bottles inoculated twice. In neither case did infection take place.
408	7.31	7.31	0.00	0.00			
95	7.17	7.17	0.00	0.00			
579	7.23	7.22	0.01	0.14			
644	7.54	7.54	0.00	0.00			
610	7.31	7.31	0.00	0.00			
154	7.53	7.53	0.00	0.00	0.01		
1051	9.37	9.37	0.00	0.00		Control	Error due to weighing ± 0.02 per cent.
1052	8.53	8.53	0.00	0.00			
1053	9.11	9.11	0.00	0.00			
1054	8.60	8.60	0.00	0.00			
1055	7.32	7.32	0.00	0.00			
1056	7.47	7.46	0.01	0.13			
1057	9.23	9.23	0.00	0.00			
1058	6.04	6.04	0.00	0.00			
1059	8.53	8.53	0.00	0.00			
1060	9.16	9.15	0.01	0.11	0.02		

BACTERIA IN RELATION TO CELLULOSE FERMENTATION AS INDICATED BY THE DECAY OF WOOD

Isolation of bacteria present on decaying wood under natural conditions and the determination of their cellulose-dissolving properties.—It has been satisfactorily demonstrated by numerous investigators that certain bacteria have the power to dissolve cellulose under both aërobic and anaërobic conditions. If these forms play any part in the decay of wood under natural conditions they should be found present in connection with the fungi or alone on decaying wood. To determine this the bacteria from numerous samples of decaying wood were isolated and their cellulose-dissolving properties investigated.

Method of isolation.—Small samples of decaying wood were placed in test-tubes containing about 10 cc. of sterile distilled water and allowed to soak from five to ten minutes with frequent vigorous shakings of the tube to facilitate removing any bacteria which might adhere to the surface of the samples. Transfers were made from the water blanks to tubes of melted media and plated in the usual manner.

The following kinds of media were employed: hard potato agar, cotton cellulose agar, filter-paper cellulose agar, oak cellulose agar, ash cellulose agar, Douglas fir cellulose agar, and western hemlock cellulose agar.

The hard potato agar had the following composition:

Extract from	200 gms. potato
Glucose	20 gms.
Agar	20 gms.
Distilled water to make	1000 cc.

The filter-paper cellulose was prepared in the manner described by McBeth and Scales ('13) and later by Cooley ('14). Fifteen gms. of filter-paper were dissolved in Schweitzer's solution and precipitated with hydrochloric acid. The mixture was diluted to ten liters and the cellulose allowed to settle. After settling, the supernatant liquid was poured off and the cellulose thoroughly washed with hydrochloric acid to get rid of all traces of copper. It was then thoroughly washed with

distilled water to remove all traces of chlorine. The water was filtered off with a Buchner's funnel and the cellulose washed repeatedly. After washing, it was transferred to a liter flask containing 500 cc. of distilled water, and sterilized.

Cotton cellulose was prepared in the same manner as the above.

The wood cellulose was prepared by treating a quantity of fine shavings of the wood in question with a solution composed of 30 gms. of potassium chlorate dissolved in 520 cc. of nitric acid (sp. gr. 1.1). The shavings were added slowly to the solution while the flask was immersed in ice water to prevent excessive heating. After the addition of the shavings the mixture was kept cool for four to five weeks when the shavings were well washed and then dissolved in Schweitzer's solution in the same manner as was the filter-paper and the cotton.

Cellulose agar was prepared by adding about 1 per cent (by weight) of the precipitated celluloses to a mineral nutrient solution having the following composition:

Monopotassium phosphate	1 gm.
Magnesium sulphate	1 gm.
Sodium chloride	1 gm.
Ammonium sulphate	1 gm.
Calcium carbonate	2 gms.
Distilled water	1000 cc.

Twenty gms. of agar were added to a mixture containing 500 cc. of the above solution and 500 cc. of the cellulose suspension.

It is evident that the cellulose present in media made up in this manner is the only source of carbon. Growth of an organism, therefore, on the medium may be taken as an indication of the power to utilize cellulose. Table XII indicates the results of the attempts to isolate cellulose-dissolving organisms from decaying wood. The cellulose agar plates were kept under observation from two to three weeks at room temperature.

In no case was a bacterial form found on any of the cellulose agars. The fact that none developed, however, does not preclude the possibility of their presence on the decaying wood. It is a known fact that some organisms require the presence

TABLE XII

ISOLATION OF CELLULOSE-DISSOLVING BACTERIA FROM DECAYING WOOD

Sample no.	Place of collection	Date of collection	Kind of wood	No. of bacterial colonies on Thaxter's agar	Number of colonies on cellulose agar					
					Filter paper	Cotton	Ash	Oak	Douglas fir	Western hemlock
1	St. Louis	10/ 3/16	Willow	0	0	0	0	0	0	0
2		10/ 3/16	Willow	8	0	0	0	0	0	0
3		11/24/16	Locust	4	0	0	0	0	0	0
4		11/24/16	Locust	6	0	0	0	0	0	0
5		11/24/16	Ash	3	0	0	0	0	0	0
6		11/24/16	Osage orange	9	0	0	0	0	0	0
7		11/24/16	Juniper	2	0	0	0	0	0	0
8		11/24/16	Oak	3	0	0	0	0	0	0
9		11/24/16	Oak	2	0	0	0	0	0	0
10		11/27/16	Palm stem	5	0	0	0	0	0	0
11	Seattle	10/10/16	Douglas fir	2	0	0	0	0	0	0
12		10/10/16	Douglas fir	0	0	0	0	0	0	0
13		10/10/16	Douglas fir	0	0	0	0	0	0	0
14		10/10/16	Douglas fir	0	0	0	0	0	0	0
15		10/10/16	Douglas fir	3	0	0	0	0	0	0
16		10/10/16	Douglas fir	7	0	0	0	0	0	0
17		10/10/16	Douglas fir	4	0	0	0	0	0	0
18		10/10/16	Douglas fir	6	0	0	0	0	0	0
19		10/10/16	Western hemlock	7	0	0	0	0	0	0
20		10/10/16	Western hemlock	0	0	0	0	0	0	0
21		10/10/16	Western hemlock	2	0	0	0	0	0	0
22		10/10/16	Western hemlock	3	0	0	0	0	0	0
23		10/10/16	Western hemlock	0	0	0	0	0	0	0
24		10/10/16	Western hemlock	1	0	0	0	0	0	0
25		10/10/16	Western hemlock	3	0	0	0	0	0	0
26		10/10/16	Western hemlock	3	0	0	0	0	0	0
27		10/10/16	Western hemlock	2	0	0	0	0	0	0
28		10/10/16	Western hemlock	1	0	0	0	0	0	0
29		10/10/16	Western hemlock	0	0	0	0	0	0	0
31		10/10/16	Western hemlock	4	0	0	0	0	0	0
32	St. Louis	10/10/16	Ash	1	0	0	0	0	0	0
33		10/10/16	Ash	4	0	0	0	0	0	0
34		10/10/16	Maple	3	0	0	0	0	0	0
35		10/10/16	Maple	5	0	0	0	0	0	0

of small quantities of other carbohydrates in their early stages of development before cellulose can be utilized. In many of the plates species of *Penicillium* developed which dissolved the cellulose to a considerable extent. This could be determined by the clearing up of the agar near the colonies. That some species of *Penicillium* have this power has been shown by Ward ('98) and McBeth and Scales ('13).

RELATION OF BACTERIA TO THE RATE OF DECAY INDUCED BY FUNGI UNDER LABORATORY CONDITIONS

It is possible that even though the cellulose-dissolving bacteria play no part in the decay of wood under natural conditions, the ordinary air, soil, and water forms, so universally found in connection with decaying wood, may have an important indirect influence on the rate of decay caused by fungi. This may come about by these organisms changing the reaction of the substratum in such a manner that it may be more or less favorable to the optimum development of the fungus; or it is also quite possible that in their metabolism they may utilize some of the hydrolytic products produced by the fungus, an accumulation of which might be partially toxic to the fungus. In order to determine whether or not this is true, pure cultures of bacteria were added to pure cultures of different fungi and their effect, if any, on the rate of decay noted.

Methods and materials.—Approximately equal amounts of sawdust were placed in clean weighed 125-cc. wide-necked bottles. The bottles and contents were placed in a hot-air oven and dried to constant weight at a temperature of 90° C. and weighed again. Approximately equal amounts of water were added to each flask and they were then plugged and sterilized at fifteen pounds for twenty minutes. Every effort was made to have conditions as nearly identical as possible in each bottle. Each was inoculated with a wood-destroying fungus, and after the wood was well infected bacteria were added and the rate of decay determined by the loss in weight of the culture bottles after six months.

The experiments were conducted with four species of commercial woods, Douglas fir (*Pseudotsuga taxifolia*), western hemlock (*Tsuga heterophylla*), white ash (*Fraxinus sp.*), and red oak (*Quercus sp.*). Heart-wood was used in each instance. The Douglas fir and western hemlock wood were obtained from freshly sawed logs. The white ash and red oak were purchased from lumber dealers and were in all probability air-seasoned. The sawdust was prepared as previously discussed.

CULTURE RELATIONS: FUNGI

The three fungi used were selected because of their vigorous growth in cultures, their importance as wood-destroying forms, and the fact that they are found on both coniferous and deciduous hosts. *Fomes pinicola* Fr. is particularly destructive on a great variety of conifers. It has also been reported by Weir ('14) on *Populus trichocarpa, P. tremuloides, Betula occidentalis, Fagus sylvatica, Quercus pendiculata,* and other deciduous hosts. *Polystictus versicolor* (L.) Fr. has a wide range of deciduous hosts and has also been reported by Weir ('14) on *Larix occidentalis, Pinus monticola,* and *Pseudotsuga taxifolia. Lenzites saepiaria* Fr. has been previously used in this laboratory by Zeller ('16) with excellent results. It is more important as an organism causing decay of coniferous woods but has also been reported on *Populus alba, P. tremuloides, Betula occidentalis, Acer glabrum,* and other deciduous hosts by Weir ('14).

Cultures of *Fomes pinicola* were made from young sporophores by the tissue method first reported by Duggar ('05) and later used by Zeller ('16) in obtaining cultures of *Lenzites saepiaria.* Young sporophores, from four to six inches in diameter, were washed with tap water followed by a thorough washing with sterile distilled water, and then dried with sterile tissue towelling. An incision from one-half to one centimeter deep was made across both surfaces of the sporophore with a sterile scalpel and the sporophore then broken open. This eliminated the possibility of contamination of the deeper tissue by the carrying down by the scalpel of bacteria and spores from the surface of the sporophore and also left a jagged surface on the broken deeper tissues, portions of which were easily torn off by means of a sterile forceps and quickly transferred to Thaxter's hard agar slants. The fungus grew exceptionally well on this media. Twenty-four tissue transfers were made, of which twenty-three grew, none being contaminated.

Cultures of *Lenzites* and *Polystictus* were made by the spore method. This method has been extensively used by Falck ('02), Lyman ('07), Münch ('09), and Zeller ('16). The tech-

nique involved in making spore cultures of the two forms is similar. The sporophores were thoroughly rinsed with sterile distilled water to remove as many bacteria and foreign spores of fungi as possible. Since gentle agitation facilitates this, it was found convenient to place the sporophores in sterile wide-necked flasks partially filled with sterile distilled water. After a gentle shaking the sporophores were removed with a pair of sterile forceps, dried with sterile tissue towelling and placed in other similar flasks. By repeating this process three times the surface of the sporophore is found to be comparatively free from bacteria and the spores of foreign fungi. Following this preliminary washing the sporophores were allowed to soak from one to two hours in sterile distilled water, then thoroughly rinsed and dried as before, and finally placed hymenium downward in large sterile Petri dishes. By means of short sterile glass rods the sporophore was kept from resting on the bottom of the Petri dish and thus the possibility of contamination of the Petri dish by contact with the sporophore was avoided. In from twelve hours to two days the spores were discharged to such an extent that they were plainly visible on the bottom of the Petri dish. By means of a platinum loop they were removed to a sterile water blank and by the same method were transferred from the water blank to tubes of molten agar and then plated in the usual manner. Transfers were made from characteristic colonies from the plates to agar slants.

A much easier and quicker method, though less certain to be a pure culture, is to place the washed sporophores in Petri dishes containing a small amount of sterile agar. The sporophores are not allowed to rest directly on the agar but are kept from it by means of short sterile glass rods lying across the agar. The spores thus fall directly on the nutrient agar and after germination transfers can be made directly to agar slants. Cultures free from contamination were obtained by this method but were not used on account of the decided advantages of the other method.

In order to avoid growing the fungi on artificial media for any length of time before using them in the decay experiments,

they were transferred to sterile Douglas fir and hemlock sawdust in Erlenmeyer flasks for keeping. All three forms grew comparatively well on both kinds of sawdust.

Optimum degree of acidity of media for fungi used.—In connection with this work it was necessary to determine whether or not slight changes in the reaction of the medium had any influence on the rate of growth of the fungi. Media of different degrees of acidity were therefore made up. Three liters of medium having the following composition per liter were prepared:

Extract from 450 gms. carrot	
Glucose	10 gms.
Agar	25 gms.
Distilled water to make..	1000 cc.

Two-hundred-cc. amounts of this medium were placed in 500-cc. Erlenmeyer flasks and autoclaved for twenty minutes at fifteen pounds. This medium titrated +13.5 Fuller's scale after autoclaving. After adding varying amounts of sterilized hydrochloric acid or sodium hydroxide to the flasks samples were again titrated while the agar was still in a liquid condition. Twenty-cc. amounts were placed in each of six Petri dishes of approximately nine centimeters diameter. Before inoculating these plates with the fungi they were allowed to stand for three days at room temperature in order to determine whether or not they were sterile. For each fungus two plates were used for each concentration. The colonies were measured in two directions at right angles to each other and the average taken.

It was endeavored to inoculate all plates in a practically identical manner, and to do this, plate cultures of the fungus in question were cut by means of a sterile scalpel into squares measuring approximately three-quarters of a centimeter on each side. A single square was placed in the center of each plate and after six and again after eight days' incubation at room temperature the size of the colonies was measured. The results are tabulated in table XIII:

TABLE XIII

RATE OF GROWTH OF FOMES PINICOLA, POLYSTICTUS VERSICOLOR, AND LENZITES SAEPIARIA ON MEDIA OF INCREASING ACIDITY

Reaction of media, Fuller's scale	Diameter of colonies in centimeters					
	Fomes pinicola		Polystictus versicolor		Lenzites saepiaria	
	6 days	8 days	6 days	8 days	6 days	8 days
+ 2.5	No growth	1.8	No growth	1.6	No growth	2.3
+ 4.5	1.8	3.0	4.9	7.0	2.3	3.7
+ 6.0	1.9	3.1	5.4	7.6	2.4	3.9
+ 7.75	2.0	3.1	6.3	8.3	2.4	3.9
+ 9.75	1.9	3.1	6.6	9.3	3.2	4.1
+13.5	2.1	3.3	5.7	7.4	2.4	4.0
+14.0	1.9	3.1	5.8	7.4	2.5	4.0
+16.25	1.8	2.9	5.3	6 9	2.6	3.9
+18.5	1.7	2.9	5.1	6.4	2.7	4.1
+21.25	1.8	3.0	4.7	6.0	2.5	3.9
+22.25	1.6	3.0	4.4	5.7	2.3	3.3
+24.5	1.7	3.0	4.3	4.6	2.1	2.8

There is but little difference in the rate of growth of *Fomes pinicola* on media ranging from + 5 to + 24.5 acid, Fuller's scale. On a medium of + 2.5 there is a decided retardation in the rate of growth. *Polystictus versicolor* shows a marked response to the reaction of the medium. A maximum is attained when the medium titrates + 9.75, Fuller's scale, with a steady decrease in the rate of growth as the acidity increases. The effect of the reaction of the medium on the rate of growth of *Lenzites saepiaria* is quite similar to that on *Fomes pinicola* except that there is a marked retardation on the media of the higher concentrations.

It is appreciated that by titrating samples of acidified agar in this manner the actual acidity of the medium is not definitely determined, due to the buffer effect of the agar on the hydrogen ions; yet the results would indicate that slight variations in the reaction of the substratum would have little or no effect on the rate of development of *Fomes pinicola* or *Lenzites saepiaria* but that they might have in the case of *Polystictus versicolor*.

CULTURE RELATIONS: BACTERIA

The bacteria used were chosen, with one exception, with reference to their acid or alkaline reactions on culture media. The presence of a nitrogen-fixing soil form was also tried. The

nomenclature used is that of Migula ('00). The following organisms were employed:

(1) Alkaline on litmus milk.
Bacillus vulgatus (Flügge) Mig.
Bacterium mycoides (Scholl) Mig.
(2) Acid on litmus milk.
Bacillus vulgaris (Hauser) Mig.
Bacillus coli (Escherlich) Mig.
Bacillus prodigiosus (Ehrenberg) Flügge.
(3) Nitrogen-fixing form.
Azotobacter chroococcum Beijerinck.

All forms were plated in the usual manner, to make certain that pure cultures were being considered, and then transferred to standard beef broth.

Method of inoculation of culture bottles.—Transfers from the sawdust cultures of the fungi were made to sterile agar plates. When the entire surface of the plate was overgrown with the mycelium it was cut into small squares of approximately one square centimeter size by means of a sterile scalpel. A single square was added to each culture bottle. In most cases the mycelium could be seen growing through the sawdust about one week after inoculation.

When the fungus mycelium had become well established in the sawdust the culture bottles were inoculated a second time either with another fungus or with bacteria. Previous to this second inoculation all culture bottles were carefully inspected and those in which the infection of the sawdust did not occur were thrown out. The second fungus inoculation was made in a similar manner to the first. The bacteria were added by transferring a considerable number of the organisms from hard agar slants to 250-cc. Erlenmeyer flasks containing distilled water. Where more than one bacterium was added to the culture bottles a mixture of the organisms was made in the same way. Approximately one cc. of bacterium suspension was added to each culture bottle. After all of the culture bottles were inoculated with the bacterial suspension transfers were made to agar slants to make certain that the action of the

distilled water had not killed the organisms. In the case of the controls and bottles which contained the fungi alone a similar amount of sterile distilled water was added so that the moisture conditions in all the culture bottles would be the same.

After all of the culture bottles were inoculated with the desired organisms the cotton plug of each was covered with two thicknesses of paraffined tissue paper to avoid excessive evaporation from the flasks, the paper being secured by means of a rubber band. This did not make an air-tight covering, but in most cases the cultures remained sufficiently moist without watering throughout the period of incubation. In the few cases in which additional watering was necessary effort was made to keep the moisture conditions similar to those of the other culture bottles.

The cultures were then removed to a very humid rotting-pit with a temperature varying from 22° C. in summer to 35° C. in winter. The period of incubation in all cases was six months.

At the end of the period of incubation small particles of sawdust, such as would adhere to a moist sterile platinum needle were transferred to sterile potato agar slants in order to determine whether or not the bacteria were still alive. The plugs were then removed and the culture bottles again dried to constant weight in the hot-air oven at a temperature of 90° C., weighed, and the loss in weight determined.

In all cases the rate of decay is based on the loss in weight of the culture bottles during the period of incubation. Each set originally contained ten cultures but some were thrown out, due to infection of the fungus not taking. The average loss in each set was determined and taken as the basis of comparison between the various sets.

DESCRIPTION OF CULTURE SERIES

Four series of cultures were prepared: series A, of red oak; series B, of white ash; series C, of western hemlock; and series D, of Douglas fir.

Culture bottles from which transfers were made to determine the presence or absence of bacteria at the end of the

period of incubation are indicated by the symbols designated in the footnotes in the following tables:

TABLE XIV (Series A)

THE EFFECT OF BACTERIA ON THE RATE OF DECAY INDUCED BY FOMES PINICOLA, POLYSTICTUS VERSICOLOR, AND LENZITES SAEPIARIA ON RED OAK

I	II	III	IV	V	I	II	III	IV	V
Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)	Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)
Control, error due to weighing ±0.02%					*F. pinicola* 2/16/17, *L. saepiaria* 3/30/17				
71	8.37	8.36	0.01	0.12	21	9.52	9.50	0.02	0.21
72	7.98	7.98	0.00	0.00	22	9.79	9.75	0.04	0.40
73	7.41	7.41	0.00	0.00	23	.....	.....	.....	.....
74	9.00	9.00	0.00	0.00	24	10.05	10.04	0.01	0.10
75	8.96	8.96	0.00	0.00	25	9.53	9.49	0.04	0.60
76	9.51	9.50	0.01	0.10	26	10.30	10.25	0.05	0.48
77	7.39	7.39	0.00	0.00	27	9.69	9.67	0.02	0.21
78	7.56	7.56	0.00	0.00	28	9.32	9.30	0.02	0.21
79	8.09	8.09	0.00	0.00	29	10.15	10.14	0.01	0.10
80	9.16	9.16	0.00	0.00	30	.....	.....	.....	.....
Average	.....	.....	.....	0.02	Average	.....	.....	.....	0.29
Fomes pinicola 2/16/17					*F. pinicola* 2/16/17, *P. versicolor* & *saepiaria* 3/30/17				
1	9.44	9.41	0.03	0.32	31	9.53	9.32	0.21	2.22
2	9.47	9.47	0.00	0.00	32	8.72	8.61	0.11	1.26
3	9.45	9.45	0.00	0.00	33	9.47	9.27	0.20	2.11
4	8.71	8.70	0.01	0.11	34	10.26	10.20	0.06	0.58
5	8.87	8.84	0.03	0.38	35	9.02	8.90	0.12	1.33
6	8.83	8.83	0.00	0.00	36	9.36	9.20	0.16	1.70
7	9.51	9.48	0.03	0.31	37	9.41	9.35	0.06	0.63
8	9.03	9.03	0.00	0.00	38	9.96	9.91	0.05	0.50
9	9.34	9.33	0.01	0.11	39	9.71	9.53	0.18	1.85
10	9.00	9.00	0.00	0.00	40	9.55	9.52	0.03	0.31
Average	.....	.....	.....	0.10	Average	.....	.....	.....	1.24
F. pinicola 2/16/17, *P. versicolor* 3/29/17					*F. pinicola* 2/16/17, *B. prodigiosus* 3/30/17				
11	8.75	8.72	0.03	0.34	41	9.23	9.18	0.05	0.54
12	9.22	9.20	0.02	0.21	42	9.35	8.95	0.40	4.27
13	9.42	9.39	0.03	0.32	43	9.94	9.86	0.08	0.80
14	9.11	9.09	0.02	0.22	44	8.70	8.43	0.27	3.08
15	.....	.....	.....	.....	45	9.45	9.26	0.19	2.01
16	.....	.....	.....	.....	46	8.97	8.91	0.06	0.67
17	9.89	9.78	0.11	1.11	47*	9.12	8.99	0.13	1.42
18	9.26	9.26	0.00	0.00	48	9.42	9.20	0.22	2.46
19	9.12	9.07	0.05	0.55	49*	9.14	9.04	0.10	1.09
20	9.95	9.92	0.03	0.30	50*	9.33	9.19	0.14	1.60
Average	.....	.....	.....	0.36	Average	.....	.....	.....	1.79

* No bacteria present at end of incubation.

TABLE XIV (Continued)

I	II	III	IV	V
F. pinicola 2/16/17, *Bact. mycoides* 3/31/17				
51	9.47	9.16	0.31	3.37
52	9.36	9.15	0.21	2.23
53	10.22	10.04	0.18	1.86
54	10.15	10.00	0.15	1.08
55	9.99	9.93	0.06	0.60
56*	9.25	9.06	0.19	2.06
57	9.58	9.38	0.20	2.09
58	9.24	8.99	0.25	2.81
59*	9.37	9.14	0.13	1.38
60*	9.91	9.67	0.24	2.52
Average				2.00
F. pinicola 2/16/17, *B. prodigiosus* & *Bact. mycoides* 3/31/17				
61	9.74	9.49	0.25	2.57
62	9.90	9.69	0.21	2.12
63*	8.98	8.75	0.23	2.56
64*	9.79	9.54	0.25	2.55
65	9.59	9.49	0.10	1.04
66*	8.69	8.57	0.12	1.38
67	9.74	9.67	0.07	0.71
68	10.02	9.73	0.29	2.89
69	9.01	8.82	0.19	2.12
70	11.17	10.97	0.20	1.79
Average				1.97
Lenzites saepiaria 2/15/17				
81	9.19	9.18	0.01	0.11
82	8.87	8.78	0.09	1.01
83	8.75	8.74	0.01	0.11
84	10.73	10.72	0.01	0.09
85	10.27	10.27	0.00	0.00
86	8.99	8.98	0.01	0.11
87	8.42	8.42	0.00	0.00
88	8.61	8.60	0.01	0.12
89	9.32	9.30	0.02	0.22
90	9.45	9.44	0.01	0.11
Average				0.18
L. saepiaria 2/15/17, *B. prodigiosus* 3/30/17				
91	8.80	8.75	0.05	0.52
92*	9.35	9.27	0.08	0.85
93	9.74	9.63	0.11	1.13
94*	8.95	8.68	0.27	3.05
96	8.51	8.42	0.09	0.99
97	9.13	9.07	0.06	0.66
100*	8.40	8.35	0.05	0.59
Average				1.11

I	II	III	IV	V
L. saepiaria 2/15/17, *Bact. mycoides* 3/31/17				
102*	8.48	8.43	0.05	0.59
103	8.75	8.72	0.03	0.34
104	8.92	8.92	0.00	0.00
105	8.78	8.67	0.11	1.25
107	8.36	8.34	0.02	0.23
108*	8.64	8.64	0.00	0.00
109	8.91	8.79	0.12	1.35
110*	8.26	8.20	0.06	0.72
Average				0.56
L. saepiaria 2/5/17, *B. prodigiosus* & *Bact. mycoides* 3/31/17				
111	9.22	9.22	0.00	0.00
113*	8.57	8.57	0.00	0.00
116	9.93	9.86	0.07	0.75
117*	9.73	9.61	0.12	1.23
118	9.49	9.48	0.01	0.11
119	8.38	8.38	0.00	0.00
121	7.85	7.85	0.00	0.00
122*	8.89	8.89	0.00	0.00
Average				0.25
L. saepiaria 2/5/17, *B. vulgatus* & *Bact. mycoides* 4/2/17				
883*	10.94	10.90	0.04	0.36
885	9.66	9.55	0.11	1.14
887†‡	10.23	10.06	0.17	1.66
888†‡	9.06	9.03	0.03	0.33
889	8.80	8.78	0.02	0.23
890	8.72	8.68	0.04	0.46
Average				0.69
L. saepiaria 2/5/17, *B. vulgaris* & *B. coli* 4/2/17				
131*	10.07	10.01	0.06	0.60
132	8.51	8.46	0.05	0.59
133*	10.39	10.36	0.03	0.30
134	9.51	9.37	0.14	1.47
135	8.26	8.15	0.11	1.36
136	9.33	9.21	0.12	1.29
137	10.67	10.44	0.23	2.16
138	8.79	8.75	0.04	0.45
140*	10.04	10.02	0.02	0.20
Average				0.95

* No bacteria present after incubation.
† *Bacterium mycoides* present after incubation.
‡ *Bacillus vulgatus* present after incubation.

TABLE XIV (Continued)

I	II	III	IV	V	I	II	III	IV	V
Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)	Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)
L. saepiaria 2/5/17, *Azotobacter chroococcum* 4/2/17					*P. versicolor* 2/4/17, *Bact. mycoides* 3/30/17				
141*	9.58	9.47	0.11	1.15	172*	10.55	10.54	0.01	0.10
142	8.67	8.61	0.06	0.69	173*	9.40	9.25	0.15	1.60
143	9.82	9.73	0.09	0.92	174	10.08	9.95	0.13	1.28
144*	8.09	8.02	0.07	0.86	175	10.15	9.99	0.16	1.58
145	9.37	9.35	0.02	0.21	176	10.24	10.23	0.01	0.10
146*	8.44	8.41	0.03	0.35	177	9.18	9.14	0.04	0.44
147	8.76	8.67	0.09	1.03	178	8.07	7.98	0.09	1.11
148	10.35	10.16	0.19	1.86	179	10.11	10.11	0.00	0.00
149	8.43	8.43	0.00	0.00	180*	8.54	8.53	0.01	0.12
Average				0.78	Average				0.70
Polystictus versicolor 2/4/17					*P. versicolor* 2/4/17, *B. prodigiosus* & *Bact. mycoides* 3/31/17				
151	11.75	11.75	0.00	0.00	181	9.12	9.05	0.07	0.77
152	8.02	8.02	0.00	0.00	182*	10.34	10.31	0.03	0.28
153	11.15	11.15	0.00	0.00	183	9.88	9.88	0.00	0.00
155	9.18	9.18	0.00	0.00	184	9.27	9.14	0.13	1.40
156	10.25	10.25	0.00	0.00	185	10.11	10.09	0.02	0.20
157	7.74	7.73	0.01	0.13	186*	10.49	10.33	0.16	1.52
158	9.11	9.11	0.00	0.00	187	9.39	9.31	0.08	0.85
161	10.05	10.05	0.00	0.00	188	9.30	9.24	0.06	0.65
162	10.31	10.29	0.02	0.19	189*	9.16	9.10	0.06	0.65
					190	10.36	10.33	0.03	0.29
Average				0.04	Average				0.66
P. versicolor 2/4/17, *B. prodigiosus* 3/30/17					*P. versicolor* 2/4/17, *B. vulgaris* & *B. coli* 4/2/17				
191*	8.63	8.61	0.02	0.23	201	9.28	9.17	0.11	1.19
192	9.01	8.98	0.03	0.33	202*	9.55	9.46	0.09	0.94
193	8.59	8.57	0.02	0.23	203*	9.31	9.11	0.20	2.14
194	8.86	8.79	0.07	0.81	204	9.90	9.73	0.17	1.72
195	10.95	10.81	0.14	1.28	205*	9.70	9.69	0.01	0.10
196*	8.59	8.49	0.10	1.24	206	10.00	9.94	0.06	0.60
197	8.86	8.77	0.09	1.01	207	10.43	10.25	0.18	1.72
198	9.35	9.35	0.00	0.00	208	8.93	8.81	0.12	1.34
199*	10.02	9.66	0.36	3.53	210	9.53	9.52	0.01	0.11
Average				0.61	Average				0.98

* No bacteria present after incubation.

TABLE XIV (Continued)

I	II	III	IV	V	I	II	III	IV	V
P. versicolor 2/4/17, *Azotobacter chrococcum* 4/2/17					*P. versicolor* 2/4/17, *B. vulgatus* & *Bact. mycoides* 4/2/17				
211*	9.12	9.12	0.00	0.00	891	8.26	8.26	0.00	0.00
212*	10.48	10.41	0.07	0.67	892	10.86	10.69	0.17	1.59
213	8.46	8.41	0.05	0.59	893	9.03	8.94	0.09	1.00
214*	9.78	9.77	0.01	0.11	894	8.73	8.63	0.10	1.14
215	8.57	8.43	0.14	1.63	895†‡	9.36	9.20	0.16	1.71
216	8.59	8.56	0.03	0.35	896	10.59	10.42	0.17	1.61
217	9.25	9.12	0.13	1.40	897†‡	9.43	9.40	0.03	0.32
218	9.25	9.20	0.05	0.54	898	9.27	9.13	0.14	1.51
219	9.52	9.49	0.03	0.37	899	8.92	8.88	0.04	0.45
220	9.70	9.70	0.00	0.00	900†‡	9.11	9.08	0.03	0.33
Average				0.57	Average				0.96

TABLE XV (Series B)

THE EFFECT OF BACTERIA ON THE RATE OF DECAY INDUCED BY FOMES PINICOLA, POLYSTICTUS VERSICOLOR, AND LENZITES SAEPIARIA ON WHITE ASH

Control, error due to weighing ±0.01%					*F. pinicola* 2/16/17, *L. saepiaria* 3/30/17				
1071	9.00	9.00	0.00	0.00	681	6.06	5.78	0.28	4.61
1072	6.17	6.17	0.00	0.00	682	5.83	5.57	0.26	4.46
1073	6.29	6.28	0.01	0.15	684	6.29	5.81	0.48	7.13
1074	5.80	5.80	0.00	0.00	685	5.58	5.10	0.48	8.59
1075	7.75	7.75	0.00	0.00	686	7.63	7.45	0.18	2.36
1076	9.35	9.35	0.00	0.00	687	6.35	5.97	0.38	6.00
1077	9.01	9.01	0.00	0.00	688	7.41	7.15	0.26	3.50
1078	9.87	9.87	0.00	0.00	689	5.94	5.45	0.49	8.25
1079	8.55	8.55	0.00	0.00	690	7.60	7.56	0.04	0.53
1080	10.24	10.24	0.00	0.00	.....	.....	.....	.....	.....
Average				0.01	Average				5.05
Fomes pinicola 2/16/17					*F. pinicola* 2/16/17, *L. saepiaria* & *P. versicolor* 3/30/17				
661	6.17	5.93	0.24	3.89	691	6.76	4.78	1.98	28.29
663	6.50	5.96	0.54	6.79	692	5.53	3.75	1.78	32.15
664	5.74	5.54	0.20	3.49	693	6.52	4.40	2.12	33.52
665	6.70	6.55	0.15	2.24	694	6.31	4.64	1.67	31.41
666	6.04	5.77	0.27	4.47	696	6.07	4.10	1.97	32.46
667	7.08	7.02	0.06	0.85	698	5.67	3.70	1.97	34.75
668	6.26	5.98	0.28	4.47	700	6.61	4.76	1.85	28.40
669	6.77	6.61	0.16	2.36	.....	.....	.....	.....	.....
670	5.89	5.57	0.32	5.45	.....	.....	.....	.....	.....
Average				4.89	Average				31.54
F. pinicola 2/16/17, *P. versicolor* 3/30/17					*F. pinicola* 2/16/17, *B. prodigiosus* 3/30/17				
671	6.15	5.49	0.66	10.73	701*	7.35	7.15	0.20	2.72
672	7.54	6.91	0.63	8.35	702	6.74	6.52	0.22	3.32
673	6.71	4.26	1.45	21.57	704	6.77	6.47	0.30	4.43
674	5.97	4.25	1.72	28.85	705	6.53	6.33	0.20	3.06
675	6.80	5.14	1.66	24.42	706	6.07	5.95	0.12	1.98
676	5.74	4.33	1.41	24.60	707*	7.25	7.15	0.10	1.38
677	6.15	5.97	0.18	2.93	708	6.07	5.33	0.74	12.18
678	6.79	5.51	1.28	18.88	709	6.67	6.28	0.39	5.84
679	5.92	4.87	1.05	17.75	710*	6.64	6.29	0.35	5.27
680	6.84	4.56	1.28	18.71	.....	.....	.....	.....	.....
Average				17.68	Average				4.46

* No bacteria present after incubation.
† *Bacterium mycoides* present after incubation.
‡ *Bacillus vulgatus* present after incubation.

TABLE XV (Continued)

I	II	III	IV	V	I	II	III	IV	V
Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)	Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)
F. pinicola 2/16/17, *Bact. mycoides* 3/31/17					*L. saepiaria* 2/5/17, *B. prodigiosus* 3/30/17				
711*	5.86	5.17	0.69	11.75	751	6.95	4.58	2.37	34.10
712	5.80	5.65	0.15	2.59	752*	6.63	4.12	2.51	37.88
713*	6.63	7.50	0.13	1.96	753	6.76	4.87	1.89	27.91
714	6.77	6.44	0.33	4.87	754	6.94	4.34	2.60	37.46
716	6.12	6.04	0.08	1.31	755	6.25	4.53	1.72	27.52
717	7.25	7.08	0.17	2.34	756*	6.98	4.99	1.99	24.98
718*	5.92	5.89	0.03	0.51	757*	7.93	5.12	2.81	35.45
719	6.15	5.79	0.36	5.85	759	7.41	5.83	1.58	21.30
720	7.56	6.99	0.57	7.53	760	7.82	5.00	2.82	36.01
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average	.....	.....	.....	4.30	Average	.....	.....	.....	31.40
F. pinicola 2/16/17, *B. prodigiosus* & *Bact. mycoides* 3/31/17					*L. saepiaria* 2/5/17, *Bact. mycoides* 3/31/17				
721	7.09	6.72	0.37	5.22	761	7.77	5.84	1.93	24.85
723	7.14	6.37	0.77	10.78	763	9.10	5.58	3.52	38.72
725	7.53	7.02	0.51	6.77	764	7.93	4.92	3.01	37.98
726*	7.18	6.72	0.46	5.61	765*	7.75	4.95	2.80	36.12
727*	6.95	6.39	0.56	8.06	766	8.12	5.23	2.89	35.61
728*	6.18	6.07	0.11	1.76	768*	8.37	5.82	2.55	30.50
729	6.66	6.60	0.06	0.90	769	6.64	4.65	1.99	30.04
730	6.86	6.85	0.01	0.15	770*	7.25	4.80	2.45	33.80
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average	.....	.....	.....	4.91	Average	.....	.....	.....	33.74
Lenzites saepiaria 2/5/17					*L. saepiaria* 2/5/17, *B. prodigiosus* & *Bact. mycoides* 3/31/17				
742	5.71	3.82	1.89	33.10	771*	6.63	4.63	2.00	30.19
743	7.84	5.16	2.68	34.15	772	7.37	5.49	1.88	25.50
745	6.77	4.86	1.91	28.65	773	7.88	5.49	2.39	30.32
746	6.46	4.56	1.90	29.40	774*	7.88	5.40	2.48	31.50
748	6.54	4.57	1.97	30.15	775	6.19	4.07	2.12	34.30
750	6.31	4.09	2.22	35.01	776	6.47	4.78	1.69	26.13
.....	.....	.....	.....	.....	777	6.87	4.44	2.43	35.40
.....	.....	.....	.....	.....	780*	7.10	4.24	2.86	40.25
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average	.....	.....	.....	31.74	Average	.....	.....	.....	31.62

* No bacteria present after incubation.

TABLE XV (Continued)

I	II	III	IV	V	I	II	III	IV	V
L. saepiaria 2/5/17, *B. vulgatus* & *Bact. mycoides* 4/2/17					*P. versicolor* 2/5/17, *B. prodigiosus* 3/30/17				
971‡	12.34	7.91	4.43	35.90	821	7.41	3.64	3.77	50.75
972‡	9.72	6.44	3.28	33.75	822	6.43	3.38	3.05	47.45
973	8.01	5.10	2.91	36.15	823	7.84	3.78	4.06	51.25
974	8.61	5.67	2.94	34.10	824*	6.85	4.64	2.21	32.30
975	7.64	5.01	2.63	34.45	825	7.38	3.48	3.90	52.75
976*	7.64	5.18	2.46	32.20	826*	7.46	3.71	3.75	50.20
977	8.20	5.41	2.79	34.01	827*	6.57	3.16	3.41	51.80
978	8.48	5.60	2.88	34.00	829	6.41	3.40	3.01	46.90
979	8.08	5.61	2.47	30.55	830	6.49	3.20	3.29	50.75
980	8.43	5.71	2.72	32.23	.....	.....	.....	.....	.....
Average	.....	.....	.....	33.73	Average	.....	.....	.....	48.24
L. saepiaria 2/5/17, *B. vulgaris* & *B. coli* 4/2/17					*P. versicolor* 2/5/17, *Bact. mycoides* 3/31/17				
791	6.85	4.18	2.67	38.95	831	7.02	5.01	2.01	28.65
792	7.19	5.44	2.75	38.26	832	6.72	3.20	3.52	53.20
793*	6.69	4.21	2.48	37.08	833	7.76	4.04	3.72	48.90
794	6.52	4.04	2.48	38.00	834*	7.16	4.01	3.15	41.60
795*	7.26	4.31	2.95	40.01	835	7.53	3.91	3.62	46.75
796	6.90	4.07	1.83	26.53	836*	5.99	3.16	2.83	47.30
797	6.83	4.45	2.38	34.82	837	7.27	3.98	3.29	45.25
798*	6.83	4.33	2.50	36.60	838*	5.93	2.98	2.95	49.76
799	5.56	3.91	1.65	29.68	839	7.75	4.09	3.66	47.08
800	5.89	3.84	2.05	34.90	840	6.05	3.06	2.99	49.48
Average	.....	.....	.....	35.48	Average	.....	.....	.....	45.70
L. saepiaria 2/5/17, *Azotobacter chrococcum* 4/2/17					*P. versicolor* 2/5/17, *B. prodigiosus* & *Bact. mycoides* 3/31/17				
801	5.68	3.25	2.43	42.80	841	7.52	3.99	3.53	47.00
802	5.87	3.19	2.68	45.60	842*	6.71	3.82	2.89	43.03
803*	6.20	3.81	2.39	38.55	843	7.81	4.10	3.71	47.50
804*	6.09	3.60	2.49	40.95	844	8.19	4.38	3.81	46.60
805*	7.41	5.31	2.10	27.32	845	7.95	3.46	4.49	56.44
806	5.90	3.70	2.20	37.30	846*	6.97	3.49	3.48	49.95
807	7.70	4.52	3.18	41.25	847	7.47	3.84	3.63	48.70
808	6.84	4.32	2.52	36.85	848†	7.60	3.60	4.00	52.70
809	5.97	3.47	2.50	41.80	849	7.41	4.07	3.34	45.01
810	6.54	3.83	2.71	41.50	850	7.85	3.78	4.07	51.80
Average	.....	.....	.....	39.39	Average	.....	.....	.....	48.87
Polystictus versicolor 2/5/17					*P. versicolor* 2/5/17, *B. vulgaris* & *B. coli* 4/2/17				
811	5.31	4.06	1.25	23.53	861	7.18	3.35	3.83	53.40
812	5.10	4.43	0.67	13.13	862	7.85	3.83	4.02	51.20
813	6.42	5.22	1.20	18.70	863	7.16	3.96	3.20	44.70
814	7.91	4.26	3.65	46.08	864*	6.41	3.55	2.86	44.60
815	7.42	3.99	3.43	46.23	865	8.45	4.22	4.23	50.10
816	6.89	4.99	1.90	25.60	867*	6.79	3.54	3.25	47.95
817	7.73	5.54	2.19	28.33	868*	7.52	3.78	3.74	49.70
818	6.67	5.35	1.32	19.78	869	7.58	3.82	3.76	49.60
819	7.43	4.28	3.15	42.43	870	7.02	3.63	3.39	48.30
820	7.20	5.23	1.97	27.35	.....	.....	.....	.....	.....
Average	.....	.....	.....	29.11	Average	.....	.....	.....	48.84

* No bacteria present after incubation.
† *Bacterium mycoides* present after incubation.
‡ *Bacillus vulgatus* present after incubation.

TABLE XV (Continued)

I	II	III	IV	V	I	II	III	IV	V
Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)	Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)
P. versicolor 2/5/17, *Azotobacter chrococcum* 4/2/17					*P. versicolor* 2/5/17, *B. vulgatus* & *Bad. mycoides* 3/31/17				
871	7.03	3.39	3.64	51.75	982	8.54	4.37	4.17	48.75
872	6.59	3.23	3.36	51.10	983	7.44	3.81	3.63	48.80
873	5.59	3.02	2.57	46.00	984	8.74	4.36	4.38	50.10
874	6.33	3.37	2.96	46.75	985	8.03	3.94	4.09	50.80
875*	5.70	2.74	2.96	51.90	986*	7.63	4.03	3.60	47.15
876*	5.80	3.94	1.86	32.00	987	8.28	3.81	4.47	53.90
877	6.29	3.12	3.17	50.45	988*	7.64	5.42	2.22	29.10
878*	6.59	3.13	3.46	52.50	.....	.....	.....	.....	.....
879	7.07	3.90	3.17	44.80	.....	.....	.....	.....	.....
880	6.50	3.33	3.17	48.75	.....	.....	.....	.....	.....
Average	.....	.....	.....	47.60	Average	.....	.....	.....	46.94

TABLE XVI (Series C)

THE EFFECT OF BACTERIA ON THE RATE OF DECAY INDUCED BY FOMES PINICOLA, POLYSTICTUS VERSICOLOR, AND LENZITES SAEPIARIA ON WESTERN HEMLOCK

Control, error due to weighing ±0.02%					*F. pinicola* 2/16/17, *P. versicolor* 3/29/17				
1051	9.37	9.37	0.00	0.00	235	7.78	7.26	0.52	6.68
1052	8.53	8.53	0.00	0.00	236	6.10	5.95	0.15	2.46
1053	9.11	9.11	0.00	0.00	237	6.25	6.15	0.10	1.60
1054	8.60	8.60	0.00	0.00	239	7.31	7.08	0.23	3.15
1055	7.32	7.32	0.00	0.00	240	6.75	8.63	0.12	1.78
1056	7.47	7.46	0.01	0.13	241	6.69	6.58	0.11	1.64
1057	9.23	9.23	0.00	0.00	245	6.75	6.38	0.37	5.49
1058	6.04	6.04	0.00	0.00	.....	.....	.....	.....	.....
1059	8.53	8.53	0.00	0.00	.....	.....	.....	.....	.....
1060	9.16	9.15	0.00	0.11	.....	.....	.....	.....	.....
Average	.....	.....	.....	0.02	Average	.....	.....	.....	3.26
Fomes pinicola 2/16/17					*F. pinicola* 2/26/17, *L. saepiaria* 3/30/17				
222	7.43	7.25	0.18	2.48	246	7.52	6.46	1.06	14.62
223	4.78	5.43	0.35	7.32	247	7.88	6.29	1.59	21.90
224	7.27	7.19	0.08	1.67	248	7.45	6.67	0.78	10.48
225	6.06	5.92	0.14	2.31	249	7.25	7.12	0.13	1.79
226	7.28	6.96	0.32	4.40	250	7.48	6.73	0.75	10.05
227	7.55	7.41	0.14	1.85	251	9.60	8.63	0.97	10.10
.....	.....	.....	.....	.....	252	7.48	7.30	0.18	2.42
.....	.....	.....	.....	.....	253	7.18	7.10	0.08	1.11
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average	.....	.....	.....	3.34	Average	.....	.....	.....	10.31

* No bacteria present after incubation.

TABLE XVI (Continued)

I	II	III	IV	V	I	II	III	IV	V
F. pinicola 2/16/17, *P. versicolor* & *L. saepiaria* 3/30/17					*Lenzites saepiaria* 2/5/17				
255	7.47	6.86	0.61	8.16	301	6.96	6.33	1.63	23.40
256	6.97	6.22	0.75	10.75	302	6.25	5.58	0.67	10.73
257	8.01	7.06	0.95	11.87	303	7.22	5.81	1.41	19.51
258	7.02	6.29	0.73	10.40	304	7.94	6.06	1.88	23.70
259	7.58	6.67	0.91	12.00	305	7.52	5.93	1.59	21.12
262	8.00	7.07	0.93	11.62	306	6.66	5.53	1.13	16.95
263	7.66	6.93	0.73	9.53	307	7.17	5.56	1.61	22.41
.....	.....	.....	.....	.....	308	6.87	5.40	1.47	21.40
.....	.....	.....	.....	.....	309	7.29	5.85	1.44	19.70
.....	.....	.....	.....	.....	310	6.45	5.19	1.26	19.52
Average	.....	.....	.....	10.72	Average	.....	.....	.....	19.84
F. pinicola 2/16/17, *B. prodigiosus* 3/30/17					*L. saepiaria* 2/5/17, *B. prodigiosus* 3/30/17				
265*	6.42	6.09	0.33	5.15	311*	7.22	5.40	1.82	25.20
266	7.13	6.83	0.30	4.21	312	7.05	5.02	2.03	28.80
267	9.03	8.96	0.07	0.77	313	8.46	6.32	2.14	25.22
269	6.55	6.27	0.28	4.28	314	7.87	5.90	1.97	25.02
270*	6.84	6.75	0.09	1.32	315*	7.62	5.80	1.82	23.84
271*	9.02	8.32	0.70	7.77	316	6.42	4.86	1.56	24.30
272	7.61	7.11	0.50	6.56	317*	7.05	5.33	1.72	24.40
273	7.48	6.90	0.58	7.76	318	6.85	5.03	1.82	26.58
.....	.....	.....	.....	.....	319	7.84	6.95	0.89	11.36
.....	.....	.....	.....	.....	320	7.64	5.69	1.95	24.90
Average	.....	.....	.....	4.72	Average	.....	.....	.....	23.96
F. pinicola 2/16/17, *Bact. mycoides* 3/30/17					*L. saepiaria* 2/5/17, *Bact. mycoides* 3/31/17				
274	6.24	6.13	0.11	1.76	321	7.78	5.50	2.28	28.30
275†	7.59	7.31	0.28	3.69	322	7.53	5.55	1.98	26.31
276	7.37	7.07	0.30	4.07	323	6.94	5.38	1.56	22.52
277	6.98	6.60	0.38	5.44	324	7.32	5.41	1.91	26.10
278	7.32	6.91	0.41	5.60	325†	6.54	4.78	1.76	26.90
279	9.10	8.79	0.31	3.41	326	6.10	4.37	1.73	28.34
280†	7.48	7.10	0.38	5.09	328	6.67	5.15	1.52	22.80
281†	7.27	6.92	0.35	4.81	329†	7.36	5.45	1.91	25.92
282	8.32	7.92	0.40	4.81	330†	6.44	4.73	1.71	26.60
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average	.....	.....	.....	4.29	Average	.....	.....	.....	25.98
F. pinicola 2/16/17, *B. prodigiosus* & *Bact. mycoides* 3/31/17					*L. saepiaria* 2/5/17, *B. prodigiosus* & *Bact. mycoides* 3/31/17				
283	8.00	7.90	0.10	1.25	331	6.86	5.40	1.46	21.28
284	7.53	7.03	0.50	6.64	332†	7.43	5.72	1.71	23.00
285	7.18	6.89	0.29	4.04	333	6.49	4.89	1.60	24.66
286†	6.83	6.55	0.28	4.10	334	7.34	5.52	1.82	24.78
287†	7.95	7.54	0.41	5.15	335	8.16	6.01	2.15	26.30
288	7.10	6.75	0.35	4.92	336	7.12	5.30	1.82	25.56
289	6.45	6.17	0.28	4.45	337†	7.76	5.65	2.11	27.13
290†	7.39	7.03	0.36	4.88	338	8.32	6.28	2.04	24.51
291	7.60	7.31	0.29	3.82	339	7.34	6.05	1.29	17.58
.....	.....	.....	.....	.....	340†	7.82	5.60	1.22	15.61
Average	.....	.....	.....	4.36	Average	.....	.....	.....	23.04

* No bacteria present after incubation.
† *Bacterium mycoides* present after incubation.

TABLE XVI (Continued)

I	II	III	IV	V	I	II	III	IV	V
Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)	Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)
L. saepiaria 2/5/17, *B. vulgaris* & *B. coli* 4/2/17					*Polystictus versicolor* 2/5/17				
351	6.97	5.11	1.86	26.70	371	7.19	7.10	0.09	1.25
352	7.31	5.39	1.92	26.28	372	6.90	6.79	0.11	1.59
353	7.66	5.69	1.97	25.80	373	7.82	7.67	0.15	1.92
354	7.19	5.23	1.96	27.28	374	7.05	7.03	0.02	0.28
355*	6.90	5.35	1.55	22.50	375	7.29	7.16	0.13	1.78
356	6.95	5.25	1.70	24.48	376	9.92	9.81	0.11	1.11
357*	7.08	5.35	1.73	24.42	377	8.53	8.40	0.13	1.52
358	7.95	5.87	2.08	26.18	379	8.62	8.54	0.08	0.93
359*	7.55	5.51	2.04	27.03	380	9.17	8.99	0.18	1.96
360	6.96	5.33	1.63	23.40	.....	.....	.....	.....	.....
Average	.....	.....	.....	25.41	Average	.....	.....	.....	1.37
L. saepiaria 2/5/17, *Azotobacter chrococcum* 4/2/17					*P. versicolor* 2/5/17, *B. prodigiosus* 3/30/17				
361	7.54	5.62	1.92	25.48	381	8.23	7.84	0.39	4.24
362	6.36	5.49	1.87	29.45	382	7.43	7.24	0.19	2.55
363	7.56	5.53	2.03	26.86	384	6.76	6.45	0.31	4.59
364	6.52	5.83	1.69	25.91	385*	8.19	7.94	0.25	3.06
365	7.89	5.65	2.24	28.40	386	7.01	6.59	0.42	6.00
366*	7.77	5.69	2.08	26.76	387*	9.63	9.18	0.45	4.67
367	8.97	6.61	2.36	26.26	388	7.66	7.25	0.41	5.35
368	7.33	5.27	2.06	28.10	389*	8.40	7.98	0.42	5.00
369*	7.08	5.46	1.62	22.88	.....	.....	.....	.....	.....
370*	7.23	5.26	1.97	27.26	.....	.....	.....	.....	.....
Average	.....	.....	.....	26.74	Average	.....	.....	.....	4.43
L. saepiaria 2/5/17, *B. vulgatus* & *Bact. mycoides* 4/2/17					*P. versicolor* 2/5/17, *Bact. mycoides* 3/30/17				
911	6.82	5.19	1.63	23.92	392	8.78	7.38	0.40	4.56
912*	7.14	5.38	1.76	24.70	393†	8.29	8.05	0.24	3.18
913	9.08	7.04	2.04	22.48	394	7.22	6.87	0.35	4.85
914*	7.15	5.17	1.98	27.72	395†	6.62	6.46	0.16	2.42
915	7.10	5.38	1.72	24.25	397	7.60	7.17	0.43	5.66
916	7.87	5.84	2.03	25.85	398	6.39	6.26	0.13	2.04
917*	7.64	5.91	1.73	22.64	399	7.53	7.36	0.17	2.26
918	7.58	5.21	2.37	31.32	400†	7.39	7.22	0.17	2.30
919	7.79	5.75	2.04	26.23	.....	.....	.....	.....	.....
920	7.86	5.78	2.08	26.45	.....	.....	.....	.....	.....
Average	.....	.....	.....	25.55	Average	.....	.....	.....	3.41

* No bacteria present after incubation.
† *Bacterium mycoides* present after incubation.

TABLE XVI (Continued)

I	II	III	IV	V	I	II	III	IV	V
P. versicolor 2/5/17, *B. prodigiosus* & *Bact. mycoides* 3/30/17					*P. versicolor* 2/5/17, *Azotobacter chroococcum* 4/2/17				
401†	8.29	8.04	0.25	3.02	431	7.43	7.28	0.15	2.02
402	8.19	8.05	0.14	1.71	432	6.41	6.32	0.09	1.40
403	7.10	6.90	0.20	2.82	433	8.28	8.13	0.15	1.81
404	8.65	8.30	0.35	4.05	435	7.38	7.19	0.19	2.57
405	6.27	6.03	0.24	3.82	436*	7.57	7.22	0.35	4.62
406	7.13	6.97	0.16	2.24	437*	7.09	6.89	0.20	2.53
407	7.75	7.56	0.19	2.45	438	7.68	7.60	0.08	1.04
409†	7.14	6.95	0.19	2.66	439*	8.46	8.22	0.24	2.83
410†	8.86	8.68	0.18	2.03	440	8.35	8.11	0.24	2.87
Average				2.47	Average				2.41
P. versicolor 2/5/17, *B. vulgaris* & *B. coli* 4/2/17					*P. versicolor* 2/5/17, *B. vulgatus* & *Bact. mycoides* 4/2/17				
421	7.64	7.49	0.15	1.96	921	7.56	7.34	0.22	2.91
422*	8.09	7.81	0.28	3.46	922†	6.76	6.60	0.16	2.37
423*	9.84	7.36	0.48	4.48	923	6.79	6.66	0.13	1.92
424	8.02	7.68	0.34	4.23	924†	7.11	6.91	0.20	2.81
425	7.63	7.44	0.19	2.49	925	6.98	6.77	0.21	3.01
426*	6.47	6.36	0.11	1.70	926	7.18	7.02	0.16	2.23
427	7.30	7.15	0.15	2.06	927	7.00	6.75	0.25	3.58
428	7.45	7.25	0.20	2.68	928	6.46	6.25	0.21	3.24
429	7.33	6.97	0.36	4.90	929†	7.36	7.11	0.25	3.39
Average				3.15	Average				2.83

TABLE XVII (Series D)

THE EFFECT OF BACTERIA ON THE RATE OF DECAY INDUCED BY FOMES PINICOLA, POLYSTICTUS VERSICOLOR, AND LENZITES SAEPIARIA ON DOUGLAS FIR

Control, error due to weighing ±0.03%					*F. pinicola* 2/16/17, *P. versicolor* 3/29/17				
1061	7.29	7.29	0.00	0.00	451	8.25	7.82	0.43	5.21
1062	7.32	7.32	0.00	0.00	452	7.24	7.15	0.09	1.24
1063	7.09	7.09	0.00	0.00	453	7.08	6.92	0.16	2.26
1064	8.86	8.86	0.00	0.00	454	7.56	7.18	0.38	5.01
1065	9.08	9.07	0.01	0.11	455	7.65	7.63	0.02	0.26
1066	9.18	9.18	0.00	0.00	456	7.60	7.34	0.26	3.42
1067	8.07	8.06	0.01	0.12	457	6.99	6.94	0.05	0.71
1068	8.46	8.46	0.00	0.00	458	7.73	7.40	0.33	4.27
1069	7.21	7.21	0.00	0.00	459	7.58	7.15	0.43	5.68
1070	9.28	9.29	0.01	0.11					
Average				0.03	Average				3.12
Fomes pinicola 2/16/17					*F. pinicola* 2/16/17, *L. saepiaria* 3/30/17				
441	7.62	7.11	0.51	6.68	461	8.32	8.05	0.27	3.24
442	8.55	8.00	0.55	5.75	463	6.43	6.35	0.08	1.24
443	7.25	7.18	0.07	0.96	464	8.02	7.77	0.25	3.12
444	7.72	7.65	0.07	0.91	465	7.62	7.50	0.12	1.58
445	8.25	7.75	0.50	6.05	466	7.39	7.04	0.35	4.75
446	7.57	7.45	0.12	1.58	467	7.78	7.56	0.22	2.83
447	8.04	7.73	0.31	3.86	469	7.06	6.67	0.39	5.50
448	8.01	7.83	0.18	2.25	470	8.16	7.76	0.40	4.90
449	8.04	7.94	0.10	1.24					
450	6.84	6.81	0.03	0.44					
Average				2.97	Average				3.02

* No bacteria present after incubation.

† *Bacterium mycoides* present after incubation.

TABLE XVII (Continued)

I	II	III	IV	V	I	II	III	IV	V
Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)	Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)
F. pinicola 2/16/17, *L. saepiaria* & *P. versicolor* 3/30/17					*F. pinicola* 2/16/17, *B. prodigiosus* & *Bact. mycoides* 3/31/17				
471	8.41	7.81	0.60	7.13	501	8.84	8.40	0.44	4.98
472	8.62	7.78	0.84	9.75	502	8.52	8.18	0.34	3.99
473	8.18	7.55	0.63	7.71	503	8.69	8.47	0.22	2.53
474	8.23	7.64	0.59	7.18	504	9.22	8.87	0.35	3.69
475	9.10	8.59	0.51	5.61	505	8.98	8.56	0.42	4.67
476	7.42	7.00	0.42	5.66	506†	8.21	7.71	0.50	6.09
478	7.45	6.88	0.57	7.65	507	7.77	7.31	0.46	6.28
479	8.74	8.18	0.56	6.43	508†	7.89	7.67	0.22	2.79
480	8.45	7.56	0.89	10.52	509†	7.89	7.72	0.17	2.16
					510	9.86	9.40	0.46	4.65
Average				7.52	Average				4.18
Fomes pinicola 2/16/17, *B. prodigiosus* 3/30/17					*Lenzites saepiaria* 2/5/17				
481	9.51	8.89	0.62	6.52	521	7.87	6.96	0.91	11.56
482	9.91	9.51	0.40	4.04	522	9.49	8.12	1.37	14.45
483	10.36	9.87	0.49	4.74	523	8.39	7.22	1.17	13.98
484	9.31	8.65	0.66	7.08	524	7.56	6.65	0.91	12.01
485*	7.37	7.19	0.18	2.46	525	7.67	6.60	1.07	13.95
486	9.54	8.92	0.62	6.50	526	7.91	6.66	1.25	15.80
487*	8.83	8.36	0.47	5.32	528	7.61	6.57	1.04	13.65
488	8.35	8.01	0.34	4.07	529	9.38	8.12	1.26	13.42
489*	7.45	7.20	0.25	3.36	530	9.16	7.69	1.47	16.02
490	8.25	7.85	0.35	4.24					
Average				4.83	Average				14.98
F. pinicola 2/16/17, *Bact. mycoides* 3/31/17					*L. saepiaria* 2/5/17, *B. prodigiosus* 3/31/17				
491	8.48	8.03	0.45	5.31	531	7.81	6.63	1.18	15.09
492	8.66	8.23	0.43	4.96	532*	8.67	7.36	1.31	15.13
493*	7.86	7.62	0.24	3.05	533	6.73	5.71	1.02	15.18
494	10.72	10.27	0.45	4.19	534*	8.01	6.54	1.47	18.38
495	7.63	7.45	0.18	2.36	536	7.43	6.21	1.22	16.42
496	9.26	8.71	0.55	5.92	537	7.51	6.17	1.34	17.83
497*	7.04	6.78	0.26	3.70	538*	8.58	7.00	1.58	18.45
498	10.16	9.84	0.32	3.14	539	7.44	5.98	1.46	19.61
499	7.65	7.42	0.23	3.01	540	9.53	8.15	1.38	14.47
500*	8.26	7.85	0.41	4.96					
Average				4.06	Average				16.39

* No bacteria present after incubation.
† *Bacterium mycoides* present after incubation.

TABLE XVII (Continued)

I	II	III	IV	V	I	II	III	IV	V
L. saepiaria 2/5/17, *Bact. mycoides* 3/31/17					*L. saepiaria* 2/5/17, *B. vulgatus* & *Bact. mycoides* 4/2/17				
541	8.70	7.35	1.35	15.51	941*	8.84	7.67	1.17	13.52
542	8.31	6.89	1.42	17.09	942*	8.40	7.21	1.19	14.19
543*	7.82	6.50	1.32	16.89	945	7.73	6.61	1.12	14.49
544*	7.79	6.62	1.17	15.01	946	8.63	7.31	1.32	15.30
545	8.59	7.03	1.56	18.17	947*	8.50	8.33	0.17	2.00
546	6.87	5.64	1.23	17.89	948	6.76	5.75	1.01	14.93
547	8.01	6.83	1.18	14.73	949	6.98	6.03	0.95	13.60
548	9.72	8.32	1.40	14.41	.....	.....	.....	.....	.....
549*	6.62	5.72	0.90	13.59	.....	.....	.....	.....	.....
550	7.39	6.52	0.87	11.78	.....	.....	.....	.....	.....
Average	.....	.....	.....	15.51	Average	.....	.....	.....	12.57
L. saepiaria 2/5/17, *B. prodigiosus* & *Bact. mycoides* 3/31/17					*Polystictus versicolor* 2/5/17				
551*	8.41	6.96	1.45	17.25	592	8.69	8.68	0.01	0.12
552	7.53	6.35	1.18	15.65	593	8.64	8.63	0.01	0.12
553	8.84	7.75	1.09	14.08	599	8.75	8.73	0.02	0.23
554	7.97	6.66	1.31	16.40	600	8.69	8.67	0.02	0.23
555	7.45	6.53	0.92	12.35	603	8.17	8.16	0.01	0.12
556*	9.28	7.89	1.39	14.99	604	9.26	9.24	0.02	0.22
557	9.00	7.67	1.33	14.78	605	8.80	8.79	0.01	0.12
559	7.28	6.37	0.91	12.51	606	9.05	9.03	0.02	0.22
560†	7.89	6.81	1.09	13.71	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average	.....	.....	.....	14.75	Average	.....	.....	.....	0.17
L. saepiaria 2/5/17, *B. vulgaris* & *B. coli* 3/31/17					*P. versicolor* 2/5/17, *B. prodigiosus* 3/30/17				
570	8.47	7.49	0.98	11.55	608*	7.80	7.57	0.23	2.95
573	6.25	5.38	0.87	13.92	609	9.61	9.23	0.38	3.96
574*	7.56	6.46	1.10	14.56	611	7.27	7.15	0.12	1.65
575	9.09	7.52	1.57	17.28	612	8.13	8.01	0.12	1.47
576*	7.04	6.16	0.88	12.50	613*	7.15	7.09	0.06	0.84
577*	9.23	8.00	1.23	13.34	614	7.67	7.60	0.07	0.91
578	8.77	7.18	1.59	18.11	615	8.01	7.80	0.21	2.62
.....	.....	.....	.....	.....	616*	7.48	7.31	0.17	2.27
.....	.....	.....	.....	.....	617	9.27	8.96	0.31	3.34
.....	.....	.....	.....	.....	618	8.93	8.70	0.23	2.58
Average	.....	.....	.....	14.47	Average	.....	.....	.....	2.26
L. saepiaria 2/5/17, *Azotobacter chrococcum* 4/2/17					*P. versicolor* 2/5/17, *Bact. mycoides* 3/31/17				
580	8.30	7.16	1.14	13.75	619*	7.96	7.74	0.22	2.77
581	7.22	6.11	1.11	15.38	620*	7.45	7.33	0.12	1.61
586*	7.26	6.10	1.16	15.98	621*	7.37	7.30	0.07	0.95
587*	9.37	8.86	0.51	5.09	624	7.07	6.86	0.21	2.97
588	8.31	7.11	1.20	14.45	625	9.06	8.87	0.19	2.04
589*	6.96	5.88	1.08	15.52	626	7.69	7.64	0.05	0.65
590	7.13	6.08	1.05	14.72	627	8.91	8.89	0.02	0.22
.....	.....	.....	.....	.....	628	6.62	6.57	0.05	0.75
.....	.....	.....	.....	.....	629	8.33	8.22	0.11	1.32
.....	.....	.....	.....	.....	630	7.13	7.07	0.06	0.84
Average	.....	.....	.....	13.55	Average	.....	.....	.....	1.42

*No bacteria present after incubation.
†*Bacterium mycoides* present after incubation.

TABLE XVII (Continued)

I	II	III	IV	V	I	II	III	IV	V
Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)	Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)
P. versicolor 2/5/17, *Azotobacter chroococcum* 4/2/17					*P. versicolor* 2/5/17, *B. vulgatus* & *Bact. mycoides* 3/31/17				
651	7.79	7.78	0.01	0.13	951	8.88	8.75	0.13	1.46
653*	8.18	8.02	0.16	1.96	952*	7.59	7.53	0.06	0.79
654*	8.46	8.35	0.11	1.30	954†	8.59	7.54	0.05	0.58
656	8.53	7.51	0.02	0.23	955	8.09	8.03	0.06	0.74
657*	9.95	9.75	0.20	2.01	956	7.01	6.91	0.10	1.56
659	7.09	7.07	0.02	0.28	957*	7.16	7.06	0.10	1.39
660	7.29	7.25	0.04	0.55	959	7.41	7.29	0.12	1.62
.....	.....	.....	.....	.....	960	7.80	7.71	0.09	1.15
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average	.....	.....	.....	0.92	Average	.....	.....	.....	1.16

* No bacteria present after incubation.
† *Bacterium mycoides* present after incubation.

The average results of the foregoing series are tabulated in table XVIII.

THE GROWTH OF PURE CULTURES OF BACTERIA ON SAWDUST AND THEIR EFFECT ON THE REACTION OF THE SAWDUST EXTRACT

Due to the fact that in most of the cultures bacteria were no longer found alive at the end of the period of incubation, pure cultures of the organisms were grown on sawdust in order to determine whether or not the sawdust was toxic and the effect, if any, of the bacteria on the reaction of the extract.

Equal amounts, 5.00 gms., of sawdust were placed in 125-cc. Erlenmeyer flasks and 50 cc. of distilled water were added to each. The flasks were plugged and autoclaved for one hour and subsequently inoculated with various species of bacteria. After thirty days' incubation at room temperature transfers were made from the flasks to hard potato agar, and the reaction of the wood extract determined by titrating 5 cc. of the extracts against N/20 sodium hydroxide. Red oak, western hemlock, and Douglas fir were used in this work, with *Bacillus*

TABLE XVIII

AVERAGE LOSS PER CENT IN CULTURE SERIES A, B, C AND D

Second inoculation	Average loss (per cent)											
	Series A Red oak			Series B White ash			Series C Western hemlock			Series D Douglas fir		
	Fomes pinicola	Lenzites saepiaria	Polystictus versicolor	Fomes pinicola	Lenzites saepiaria	Polystictus versicolor	Fomes pinicola	Lenzites saepiaria	Polystictus versicolor	Fomes pinicola	Lenzites saepiaria	Polystictus versicolor
Control (not inoculated)	±0.02			±0.01			±0.02			±0.03		
Fungus alone	0.10	0.18	0.04	4.89	31.74	29.11	3.34	19.34	1.37	2.97	14.98	0.17
Polystictus versicolor	0.36	.....	.....	17.68	.....	.....	2.85	.....	.....	3.12	.....	.....
Lenzites saepiaria	0.29	.....	.....	5.05	.....	.....	10.31	.....	.....	3.02	.....	.....
Polystictus versicolor & *Lenzites saepiaria*	1.24	.....	.....	31.54	.....	.....	10.72	.....	.....	7.52	.....	.....
Bacillus prodigiosus	1.79	1.11	0.61	4.46	31.40	48.24	4.72	23.96	4.43	4.83	16.39	2.26
Bacterium mycoides	2.00	0.56	0.70	4.30	33.74	45.70	4.29	25.98	3.41	4.06	15.51	1.42
Bacillus prodigiosus & *Bacterium mycoides*	1.97	0.25	0.66	4.91	31.62	48.87	4.36	23.04	2.74	4.18	14.75	.....
Bacillus vulgatus & *Bacterium mycoides*	.....	0.69	0.96	.....	33.73	46.94	.....	25.25	2.83	.....	12.57	1.16
Bacillus vulgaris & *Bacillus coli*	.....	0.95	0.98	.....	35.48	48.84	.....	25.41	3.15	.....	14.47	.....
Azotobacter chroococcum	.....	0.78	0.57	.....	39.39	47.60	.....	26.74	2.41	.....	13.35	0.92

coli, B. vulgaris, B. prodigiosus, and *B. vulgatus* as the organisms.

The results of the titrations, expressed in degrees of acidity, Fuller's scale, are given in table XIX. All series were run in triplicate and the following results are the average of three titrations:

TABLE XIX

THE EFFECT OF PURE CULTURES OF BACTERIA ON THE REACTION OF WOOD EXTRACTS

Organism	Red oak	Western hemlock	Douglas fir
Control	13.0	2.5	3.0
Bacillus coli	13.0	2.0	3.2
Bacillus vulgaris	13.5	2.5	3.0
Bacillus prodigiosus	13.7	.....	3.5
Bacillus vulgatus	13.3	.....	3.3

In the case of the red oak extract the end point could not be exactly determined because of the dark color of the extract which might account for the slight variations in the titrations. It is evident therefore that these organisms do not change the titratable acidity of the wood extracts to any considerable extent, if at all.

Transfers made from the cultures at the end of thirty days' incubation to hard potato agar were negative in every case, indicating that the wood extracts were either toxic to, or lacked some of, the necessary substances for continued growth of the organisms.

Discussion of Results

SERIES A: RED OAK

The rate of decay of red oak was comparatively slow with the three fungi used. *Lenzites saepiaria* caused the most rapid loss in weight in the culture bottles. In cultures first inoculated with *Fomes pinicola* and subsequently with *Polystictus versicolor* and *Lenzites saepiaria* the rate of decay was greater than that caused by any of the fungi growing separately and indeed greater than the combined rate of the three. In all cases the rates of decay caused by the fungi alone were less than those to which cultures of bacteria were added. With *Fomes pinicola* the highest rate of decay was produced in cul-

ture bottles to which *Bacterium mycoides* was added; with *Lenzites saepiaria* the highest rate was in the culture bottles to which *Bacillus prodigiosus* was added; and with *Polystictus versicolor* in those to which *Bacillus vulgaris* and *B. coli* were added. The only bacteria found in the culture bottles at the end of the period of incubation were *Bacillus vulgatus* and *B. coli.*

SERIES B: WHITE ASH

The highest rate of decay caused by the fungi was that caused by *Lenzites saepiaria.* Cultures of *Polystictus versicolor* to which bacteria were added lost the most weight due to decay of any of the cultures of the entire series. Culture bottles first inoculated with *Fomes pinicola* and subsequently with *Lenzites saepiaria, Polystictus versicolor,* or both, lost less weight than those inoculated with *Lenzites saepiaria* or *Polystictus versicolor* alone. This is quite different from the results obtained with red oak. The addition of bacteria to cultures of *Fomes pinicola* caused no change in the rate of decay. In the case of *Polystictus versicolor* culture bottles to which bacteria were subsequently added lost more weight than did pure cultures of the fungus. The greatest loss in weight is found in cultures of *Polystictus versicolor* to which *Bacillus prodigiosus* and *Bacterium mycoides* were added. *Bacillus vulgatus* and *Bacterium mycoides* were the only bacteria found in the culture bottles at the end of the period of incubation.

SERIES C: WESTERN HEMLOCK

Lenzites saepiaria caused the highest rate of decay on western hemlock of any of the fungi used. Cultures first inoculated with *Fomes pinicola* and subsequently with *Polystictus versicolor* lost no more weight than those inoculated with *Fomes pinicola* alone. In *Fomes pinicola* cultures subsequently inoculated with *Lenzites saepiaria* the loss in weight was practically identical to that lost by pure cultures of *Lenzites saepiaria.* When *Fomes pinicola* cultures were subsequently inoculated with both *Lenzites saepiaria* and *Polystictus versicolor* the loss in weight was also practically the same as that caused by pure cultures of *Lenzites saepiaria.* Cul-

tures of *Polystictus versicolor* to which bacteria were added, in all cases lost from two to three times more weight than was lost by pure cultures of the fungus. *Bacterium mycoides* was the only bacterium found in the culture bottles at the end of the period of incubation.

SERIES D: DOUGLAS FIR

Lenzites saepiaria caused the most rapid rate of decay of Douglas fir. The addition of bacteria to cultures of *Fomes pinicola* and *Lenzites saepiaria* caused no apparent change in the rate of decay. Cultures of *Polystictus versicolor* to which bacteria were added lost from five to ten times as much weight as did cultures of the fungus alone. *Bacterium mycoides* was the only bacterium found in the cultures at the end of the period of incubation.

SUMMARY

The experimental results obtained may be briefly summarized as follows:

1. When wood is sterilized by autoclaving it undergoes certain changes which must be considered when using wood for experimental purposes with wood-decaying fungi. Among these changes are: (a) a change in color; (b) an increase in the amount of reducing substances in the extract; (c) an increase in the acidity of the extract; (d) an increase in the hydrogen ion concentration of the extract; (e) a change in resistance towards decay.

2. Cellulose-dissolving bacteria play no important part in the decay of wood under natural conditions.

3. The results from the decaying experiments tend to indicate that the rate of decay may be materially increased by the presence of the ordinary saprophytic bacteria.

4. The influence of bacteria on fungi with reference to the rate of decay induced by the fungi varies with the different fungi on different woods.

5. Further experimentation along this line is essential.

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STUDIES IN THE PHYSIOLOGY OF THE FUNGI

VII. Growth of Wood-Destroying Fungi on Liquid Media

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For some time this laboratory has been conducting many lines of investigation to determine various physiological factors influencing the growth and development of the wood-destroying fungi. In this connection experiments have been undertaken to determine (1) which fungi are adapted to growth on liquid media; (2) what liquid media are suitable to their growth; and (3) the influence, if any, of the hydrogen ion concentrations of the media. To this end various media were used. In three of these (Czapek's, Reed's, and Richards') the H ion concentration was made to vary by using the mono–, di–, and tribasic potassium phosphates in the different solutions.

The solutions used were as follows:

1. *Czapek's solution*[1] which contains 0.5 gm. magnesium sulphate, 1.0 gm. monobasic potassium phosphate, 0.5 gm. potassium chloride, 0.01 gm. ferrous sulphate, 2.0 gms. sodium nitrate, and 30 gms. cane sugar in 1000 cc. of water.

2. *Dunham's solution* made up according to the usual method, using 10 gms. bacto-peptone and 5 gms. sodium chloride in 1000 cc. of water.

3. *A pine decoction* prepared from the inner bark and outer sap-wood of young twigs of *Pinus Strobus*.

4. *Reed's solution*[2] for the determination of cytase, which contains 10 gms. ammonium nitrate, 5 gms. dibasic potassium phosphate, 1 gm. magnesium sulphate, and 1 gm. sodium citrate in 1000 cc. of ash-cellulose suspension in water.[3]

[1] Dox, A. W. The intracellular enzymes of Penicillium and Aspergillus. U. S. Dept. Agr., Bur. An. Ind. Bul. 120: 1–70. 1910.

[2] Reed, H. S. The enzyme activities involved in certain fruit diseases. Va. Polytec. Inst., Agr. Exp. Sta. Rept. 1911-1912: 51–77. 1913.

[3] The method of preparing pure cellulose from ash wood was that described by McBeth and Scales, U. S. Dept. Agr., Bur. Pl. Ind. Bul. 266: 1–52. 1913.

5. *Richards' solution* (*E*)[1] which contains 0.5 gm. monobasic potassium phosphate, 4 gms. potassium nitrate, 2.5 gms. magnesium sulphate, 10 gms. ammonium nitrate, 50 gms. cane sugar, and a trace of ferrous sulphate in 1000 cc. of water.

6. *Sap from Acer saccharinum.*

The following twelve fungi were employed in the experiments: *Coniophora cerebella* Pers., *Daedalea confragosa* (Bolt.) Fr., *D. quercina* (L.) Fr., *Lentinus lepideus* Fr., *Lenzites vialis* Pk., *Merulius lacrymans* (Wulf.) Fr., *M. pinastri* (Fr.) Burt, *Pleurotus sapidus* Kalchb., *Polyporus lucidus* (Leys.) Fr., *Polystictus hirsutus* Fr., *P. versicolor* (L.) Fr., and *Trametes Peckii* Kalchb.

Methods

Twenty-five-cc. quantities of the above-mentioned solutions and decoctions were pipetted into Erlenmeyer flasks (125 cc. capacity). Duplicates of these were inoculated with each fungus after the flasks were sterilized at 15 pounds pressure for 20 minutes. Control flasks of all solutions were prepared in duplicate.

Before inoculation the fungi were all grown on agar-poured plates. The nutrient agar was made up in the following manner: To 1000 cc. of potato water (from 200 gms. of peeled and sliced potato cooked for 30 minutes in the autoclave at 15 pounds pressure) were added 20 gms. of cane sugar, 10 gms. of potassium nitrate, 5 gms. of monobasic potassium phosphate, and 20 gms. of agar. After growth of the fungi the plates were cut into small squares (about 8 mm. square) which were used as inocula. Thus the amount of agar added to the cultures introduced to all a negligible amount of nutrient materials. Similar squares of agar from uninoculated poured plates were added to all control flasks. All cultural operations were performed in a transfer room which was thoroughly steamed before each operation. There were no contaminations in the 332 cultures involved in the data discussed below.

[1] Richards, H. M. Die Beeinflussung des Wachsthums einiger Pilze durch chemische Reize. Jahrb. f. wiss. Bot. 30: 665–688. 1897.

The cultures were incubated at room temperature (about 15–25° C.) during a period of 30 days.

The growth of the fungi which is reported in values of × in the table was determined as follows: The culture of maximum growth in the whole series was arbitrarily designated as 10 ×, no growth as 0, and intermediate amounts of growth as 1 ×, 2 ×, 3 ×, etc.

The H ion concentrations were determined in accordance with the methods devised by Clark and Lubs,[1] and are given in P_H exponents. In the table are given the P_H values of the control flasks, the P_H values of the solutions after the fungi had grown in them for thirty days, and the amount of change in the H ion concentration of the solutions brought about by the growth of the various fungi. If the change due to growth of the organism is toward the alkaline side from the true neutral point (P_H 7) it is designated as minus and if toward the acid side, plus.

Discussion

The data presented in the table bring out some interesting features concerning the comparative growth of the fungi involved. There is a decrease in the amount of growth of *Lenzites vialis, Daedalea confragosa, D. quercina, Trametes Peckii, Merulius lacrymans, Lentinus lepideus, Coniophora cerebella, Polyporus lucidus,* and *Polystictus hirsutus* when passing from the mono- to the tribasic potassium phosphate in Czapek's solution. In *Merulius pinastri* there is a slight increase in growth, in *Pleurotus sapidus* no variation, while the growth of *Polystictus versicolor* is irregular when passing in the same direction.

In Reed's solution there is a decrease in the growth quantities of all of the fungi, except *Pleurotus sapidus, Merulius lacrymans,* and *Coniophora cerebella,* when passing from the mono- to the tribasic potassium phosphate. In *Pleurotus sapidus* growth is equal in all cases, in *Merulius lacrymans* there is a steady increase, while in *Coniophora cerebella* it is irregular.

[1] Clark, W. M., and Lubs, H. A. The colorimetric determination of hydrogen ion concentration and its applications in bacteriology. I. Jour. Bact. 2: 1–34. 1917.

TABLE I
GROWTH OF WOOD-DESTROYING FUNGI ON LIQUID MEDIA

Fungus		Dunham's Solution	Czapek's sol. (1) with KH_2PO_4	Czapek's sol. (2) with K_2HPO_4	Czapek's sol. (3) with K_3PO_4	Reed's sol. (1) with KH_2PO_4	Reed's sol. (2) with K_2HPO_4	Reed's sol. (3) with K_3PO_4	Maple sap	Richards' sol. (1) with KH_2PO_4	Richards' sol. (2) with K_2HPO_4	Richards' sol. (3) with K_3PO_4	Pine decoction
L. vialis	Gr.*	0	2×	2×	0	3×	2×	0	3×	5×	5×	5×	0
	P_H†	7.0	3.4	6.0	8.6	5.2	6.6	7.0	7.2	2.6	2.6	3.0	5.2
	Diff.‡	0.0	+1.8	+1.0	0.0	0.0	0.0	0.0	0.0	+2.2	+3.2	+3.2	+0.2
M. pinastri	Gr.	4×	9×	9×	10×	3×	2×	1×	8×	3×	5×	8×	0
	P_H	7.6	5.8	7.4	7.4	6.2	6.8	7.0	6.2	5.4	6.8	6.6	5.4
	Diff.	−0.6	−0.6	−0.4	+1.2	−1.0	−0.2	0.0	+1.0	−0.6	−1.0	−0.4	0.0
D. quercina	Gr.	0	6×	0	0	3×	0	0	0	6×	5×	1×	0
	P_H	7.0	3.2	7.0	8.6	5.2	6.6	7.0	7.0	2.6	2.8	5.4	5.4
	Diff.	0.0	+2.0	0.0	0.0	0.0	0.0	0.0	+0.2	+2.2	+3.0	+0.8	0.0
T. Peckii	Gr.	4×	5×	2×	0	3×	2×	0	4×	3×	6×	4×	0
	P_H	7.0	4.8	6.2	8.6	5.6	6.6	2.0	6.0	3.4	3.6	5.2	5.4
	Diff.	0.0	+0.4	+0.8	0.0	−0.4	0.0	0.0	+1.2	+1.4	+2.2	+1.0	0.0
P. sapidus	Gr.	5×	1×	1×	1×	2×	2×	2×	4×	2×	4×	5×	4×
	P_H	7.0	5.0	6.4	8.6	5.2	6.6	7.0	6.6	3.6	3.4	3.4	6.0
	Diff.	0.0	+0.2	+0.6	0.0	0.0	0.0	0.0	+0.6	+0.8	+2.4	+2.8	−0.6
M. lacrymans	Gr.	1×	2×	0	0	2×	3×	4×	1×	4×	5×	6×	0
	P_H	7.0	4.6	2.0	8.6	5.6	6.6	7.0	7.0	2.8	2.8	3.0	5.4
	Diff.	0.0	+0.6	0.0	0.0	0.0	0.0	0.0	+0.2	+2.0	+3.0	+3.2	0.0
L. lepideus	Gr.	0	3×	0	0	1×	0	0	1×	2×	2×	2×	0
	P_H	7.0	4.8	6.8	8.6	5.2	6.6	7.0	7.0	3.2	4.0	5.6	5.4
	Diff.	0.0	+0.4	+0.2	0.0	0.0	0.0	0.0	+0.2	+1.6	+1.8	+0.6	0.0
D. confragosa	Gr.	1×	2×	1×	0	3×	1×	0	3×	1×	2×	3×	0
	P_H	6.8	5.0	6.8	8.4	5.6	6.6	7.0	7.6	3.0	2.8	3.4	5.4
	Diff.	+0.2	+0.2	+0.2	+0.2	−0.4	0.0	0.0	−0.4	+1.8	+3.0	+2.8	0.0
C. cerebella	Gr.	0	1×	0	0	1×	0	1×	0	3×	2×	2×	0
	P_H	7.0	5.2	7.2	8.6	5.4	6.6	7.4	7.4	2.8	3.2	3.4	5.4
	Diff.	0.0	0.0	−0.2	0.0	−0.2	0.0	−0.4	−0.2	+2.0	+2.6	+2.8	0.0
P. versicolor	Gr.	0	3×	5×	2×	5×	2×	1×	7×	3×	3×	4×	5×
	P_H	7.0	4.8	4.8	4.8	6.0	6.8	7.2	5.0	3.0	3.2	3.0	5.8
	Diff.	0.0	+0.4	+2.2	+3.8	−0.8	−0.2	−0.2	+2.2	+1.8	+2.6	+3.2	−0.4
P. lucidus	Gr.	3×	7×	2×	1×	2×	2×	0	4×	4×	5×	6×	6×
	P_H	5.0	4.2	6.6	8.6	5.6	6.6	7.0	4.2	4.2	4.4	5.0	5.2
	Diff.	+2.0	+1.0	+0.4	0.0	−0.4	0.0	0.0	+3.0	+0.6	+1.4	+1.2	+0.2
P. hirsutus	Gr.	0	2×	1×	0	3×	2×	0	4×	1×	2×	3×	5×
	P_H	7.0	4.2	4.4	8.6	5.6	6.6	7.0	4.8	3.2	3.2	3.2	5.2
	Diff.	0.0	+1.0	+2.6	0.0	−0.4	0.0	0.0	+2.4	+1.6	+2.6	+3.0	0.0
Control	P_H	7.0	5.2	7.0	8.6	5.2	6.6	7.0	7.2	4.8	5.8	6.2	5.4

* Gr. = growth; † P_H = H ion concentration; ‡ Diff. = change in H ion concentration due to growth.

There is a decrease in the amount of growth of *Daedalea quercina* and *Coniophora cerebella* when passing from the mono- to the tribasic potassium phosphate in Richards' solution. In *Merulius lacrymans, M. pinastri, Trametes Peckii, Pleurotus sapidus, Daedalea confragosa, Polystictus versicolor, P. hirsutus,* and *Polyporus lucidus* there is an increase, while in *Lenzites vialis* and *Lentinus lepideus* growth is equal in all cases.

In general, Dunham's solution and the pine decoction cannot be considered suitable media for these fungi, while maple sap compares favorably with the best solution (Richards' with the tribasic potassium phosphate).

In all of the fungi studied, except *Merulius pinastri,* the general tendency is to increase the active acidity during growth. However, there are exceptions to the general tendencies, indicating the fallacy of combining the results obtained from several fungi to draw sweeping conclusions as to a definite relation between the H ion concentration and growth of wood-destroying fungi as a group. Thus, we doubt the advisability of constructing a general curve with the average data derived from a limited number of fungi, as Meacham has done in a recent note.[1] This curve would not apply to many wood-destroying fungi, as the data in the table indicate.

The influence of the growth of the same fungi on the P_H value of different solutions is shown by a comparison of the data on Reed's and Richards' solutions. On Reed's solution containing the monobasic potassium phosphate all of the fungi made fair growth and raised slightly the P_H value; on the same solution with the dibasic potassium phosphate all of the fungi made slight growth only, and there is a tendency to raise the P_H value; while with the tribasic potassium phosphate those that grew exhibited a tendency to raise the P_H value. However, on Richards' solution with the monobasic potassium phosphate all of the fungi, with the exception of *Merulius pinastri,* lower even the very low P_H value of the solution; and

[1] Meacham, M. R. Note upon the hydrogen ion concentration necessary to inhibit the growth of four wood-destroying fungi. Science N. S. **48**: 499–500. *f. 1.* 1918.

although the P_H values of the solutions with the di- and tribasic potassium phosphates were originally greater than with the monobasic phosphate the lowering due to growth was very pronounced. This difference in the shifting of the active acidity due to growth in Reed's and Richards' solutions may be attributed to the fact that in Reed's solution the citrate radical of the sodium citrate acts as the source of carbon, thus liberating alkaline sodium compounds.

CONCLUSIONS

1. Many wood-destroying fungi are not suitable for growth experiments with liquid media.

2. With respect to the media employed and to the species studied, *Merulius pinastri, Polyporus lucidus, Polystictus versicolor, Pleurotus sapidus,* and *Trametes Peckii* grow best in the order named. Others grow well only on certain media, e. g., *Lenzites vialis, Daedalea quercina,* and *Merulius lacrymans* on Richards' solution.

3. Czapek's solution with the monobasic, and Richards' solution with the mono-, di-, and tribasic potassium phosphate proved generally to be suitable media. Thus, there is a decided indication of the desirability of selecting a specific medium for each fungus.

4. In the solutions studied the H ion concentration does not seem to be the limiting factor in growth, nor in general does it appear to be the factor (within the limits studied) which determines a desirable medium.

5. The shifting of the H ion concentration due to metabolism depends both upon the fungus and the medium.

6. No general statement can be made concerning the relation between the H ion concentrations of the culture media and the growth of wood-destroying fungi as a group.

MERULIUS IN NORTH AMERICA, SUPPLEMENTARY NOTES

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Merulius incarnatus Schw.—While recently privileged to study in the Farlow Herbarium, I found in the Curtis collection an authentic specimen of *Merulius incarnatus* Schw. from Schweinitz Herbarium in such excellent state of preservation that it corrects the error which has prevailed with regard to this species for nearly a century, and which I followed in my work on 'Merulius in North America."[1] *M. incarnatus* was published as dimidiate by Schweinitz[2] and also later by Fries[3] who had seen a specimen and emphasized the point by adding that this species is unique in its section in not being effuso-reflexed. The only dimidiate, white-spored *Merulius* which now occurs in the United States eastward from the Mississippi River is *Merulius rubellus* Peck, and this species does not range east of the Appalachian Mountains. As the descriptions of *M. incarnatus* by both Schweinitz and Fries apply better to *M. rubellus* than to the true *M. incarnatus,* there seemed no doubt that Schweinitz had collected as his type of *M. incarnatus,* in North Carolina in an isolated eastern station beyond its normal range, the fungus which has since become better known under the name *M. rubellus.*

The authentic specimen of *M. incarnatus* in Curtis Herbarium is not dimidiate, in spite of the statements by Schweinitz and Fries to the contrary, as already mentioned, but it is distinctly effuso-reflexed, with the upper surface of the broadly reflexed portion somewhat tomentose, pallid by age, and the hymenium ranging from flesh-pink to the red color of garnets, listed in Ridgway as garnet-brown and Hessian

[1] Ann. Mo. Bot. Gard. 4 : 310. 1917.
[2] Naturforsch. Ges. Leipzig Schrift. 1 : 92. 1822.
[3] Elenchus Fung. 1 : 57. 1828; Epicrisis, 500. 1838.

brown. The hymenial folds form rather deep pores, radially elongated, transversely venose, and subdivided into smaller, angular pores. The fructification required an interval of several minutes after water was applied before its broad gelatinous layer softened sufficiently for sectioning. The spores are hyaline, allantoid, 3×1 μ.

This is the common species with snow-white effuso-reflexed pileus, garnet-colored hymenium, large decompound pores, and small allantoid spores, of the eastern United States. I have hitherto regarded it as a form of *M. tremellosus,* and have cited the various collections of it under *M. tremellosus* in my paper on 'Merulius.' Collections of it run down to *M. tremellosus* by the key to species of that paper, and I am not sure that it should not be referred here. However, upon examining critically seven European specimens of *M. tremellosus* which have been distributed in exsiccati, I find that none have the red hymenium of *M. incarnatus;* hence it may be practicable to separate *M. incarnatus* from *M. tremellosus* by the red hymenium of the former. Although I am unable to point out any other feature of difference, still such differences may be observed later.

M. incarnatus is common on stumps and logs of frondose species from Massachusetts to Texas, and in Missouri. June to December.

Specimens examined:

Exsiccati: Ravenel, Fungi Car. **2**: 22.

Massachusetts: Arlington Heights, *E. A. Burt;* Cambridge, *W. G. Farlow* (in Mo. Bot. Gard. Herb., 54920).

New York: East Galway, *E. A. Burt.*

Pennsylvania: *E. Michener, 478* (in Curtis Herb., and in Mo. Bot. Gard. Herb., 4077); Carbondale, *E. A. Burt;* Trexlertown, *W. Herbst.*

Virginia: Great Falls, *J. R. Weir, 8005* (in Mo. Bot. Gard. Herb., 54932).

North Carolina: *Schweinitz,* type (in Curtis Herb.).

South Carolina: *H. W. Ravenel,* in Ravenel, Fungi Car. **2**: 22; Santee, *H. W. Ravenel* (in Curtis Herb.); Society Hill (in Curtis Herb.).

Alabama: *Peters, 428* (in Curtis Herb.); Havana, *W. Trelease* (in Mo. Bot. Gard. Herb., 4071); Montgomery County, *R. P. Burke, 59, 114* (in Mo. Bot. Gard. Herb., 18206, 19801).
Texas: *Ravenel,* Dr. Billings, 114 (in Curtis Herb.).
Missouri: Creve Coeur, *E. A. Burt* (in Mo. Bot. Gard. Herb., 54328).

M. rubellus Peck.—This is a good species, although regarded by me formerly as a synonym of *M. incarnatus* Schw. in my work on 'Merulius in North America.' The description given there on p. 310 is correct for *M. rubellus,* as are also the distribution of the species and the citations of specimens examined. *M. rubellus* should be written in place of *M. incarnatus* in the key to species; and the citations of *M. incarnatus* in the synonymy and the last sentence of the comment on this species on p. 311 should be deleted.

M. hirsutus Burt.—Additional collections of this species which may be cited are:
Mexico: Jalapa, *W. A. & Edna L. Murrill, 65* (in N. Y. Bot. Gard. Herb., and in Mo. Bot. Gard. Herb., 55238); Oaxaca, Santa Ines del Monte, Zimatlan, 3000 ft. altitude, *C. Conzatti, 1377* (in Farlow Herb.).

M. lacrymans (Wulf.) Fr.—As this species is rare in North America, the following collections are recorded as additions to the list formerly published:
Massachusetts: Boston, on floor, tables, and walls up to a height of about 4 ft. in a laboratory of Massachusetts Institute of Technology, *W. G. Farlow* (in Farlow Herb.).
New Jersey: Newfield, on damp wall, *Lucian Fish,* from Ellis Herb. (in Farlow Herb.).

M. rimosus Berk.—In my work on 'Merulius in North America,' p. 327, I regarded this species as a probable synonym of *M. niveus* but had not been able to decide positively, because the cotype could not be found in N. Y. Bot. Gard. Herb. I have examined a portion of the cotype, communicated by Ellis, now in Farlow Herb., and it differs in no respect from *M. niveus.*

SEED DISINFECTION FOR PURE CULTURE WORK

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In recent years the growing of seed plants in pure cultures for both physiological and pathological purposes has become recognized as an important research method. When parasitic fungi are being studied in relation to their hosts it is necessary to control as many conditions as possible, and especially is it essential to prevent the entrance of other parasites. Moreover, when the relations of higher plants to organic nutrient solutions are being investigated, it is usually necessary to eliminate the possible action of all microörganisms. In order to obtain plants free from bacteria and fungi many and varied methods have been advocated. It is obviously necessary to begin with pure seed or with disinfected seed. This paper deals only with the latter possibility in the establishment of pure cultures.

An examination of the literature indicates that many germicides have been tried in an effort to disinfect seed. In most cases, the investigator has selected some particular germicide, which had proved favorable in one or more cases, and has attempted to make it applicable to all seeds under all conditions. Accordingly, when others attempt to use the method under different conditions poor results are obtained and the method may be considered a failure. There is much dissatisfaction, therefore, with any single germicide as an agent which will disinfect all seed under all conditions.

The chief observation that suggested a further test of disinfectants was the difficulty experienced, at the Missouri Botanical Garden, in obtaining consistent results in regard to seedlings relatively free from contamination for certain "immunity" experiments in which the author was associated with Dr. Duggar. It was found that seeds of the same varieties grown in different parts of the country had to be treated dif-

ferently and in some cases with a different germicide. This is perhaps only what is to be expected when one realizes that various soil bacteria and fungi show a wide range of tolerance towards the same toxic agent. A good example of this is cited by Makemson ('17) in his work with *Cladosporium fulvum*. He found that the spores of this fungus would germinate in a relatively strong solution of Bordeaux mixture in a hanging-drop culture. He noted further that under the conditions just mentioned the germ tubes grew as rapidly as in the control. On the other hand, the toxicity of Bordeaux mixture for most fungi is well known.

Many species of *Fusarium* are able to grow in a 1 per cent copper sulphate solution. Young and Cooper ('17), in their work on the determination of fungicidal coefficients, found that *Endothia parasitica* was killed at a dilution of 1 to 16,000 commercial lime-sulphur solution, while it required a 2 per cent solution of copper sulphate to kill this organism, both fungicides acting for a period of 15 minutes. On the other hand, when *Glomerella cingulata* was used and the same time interval employed, a dilution of 1 to 9600 commercial lime-sulphur was necessary to kill the spores, while a 1 per cent solution of copper sulphate was lethal. Lime-sulphur, therefore, is almost twice as toxic to the spores of *Endothia parasitica* as to those of *Glomerella cingulata*. The reverse is true in the case of copper sulphate. Horn ('04) found, in his work with some of the *Phycomycetes*, that exceedingly small traces of $CuSO_4$ would prevent growth. The success of the fungicide, then, must depend upon the species of bacteria or fungus present on the seed.

The nature of the seed will affect the selection of a germicide, since the resistance of different seed is as variable as that of spores. With respect to different germicides it is known that seeds may exhibit marked differences in permeability. The moisture content of the seed will also probably modify its resistance to any injurious agent. This fact has been frequently pointed out, and recently clearly demonstrated by Coons and McKinney ('17) in their work on the treatment

of grains for smuts, using formaldehyde as the disinfectant. They found that wheat seed high in moisture content absorbed the formaldehyde vapor very rapidly and were materially injured. Many other examples might be cited which, up to the present time, have seemed to indicate that no one germicide can disinfect under all conditions.

In his studies on the comparative viability of seed, fungi, and bacteria, de Zeeuw ('12) was unable by the usual method to free seeds of a certain amount of the disinfecting agent. He believed that the adherence of the agent permitted the continuance of antiseptic action, leading to a false sense of security at the time of making the transfer of the young seedling. He therefore devised a special apparatus by which it was possible to wash the seed thoroughly in order to free it from the germicide, and at the same time it prevented possible contamination from the outside during treatment.

The object of this work is to determine the effectiveness of some of the well-known disinfectants in relation to particular groups of seeds. No new methods have been devised nor new germicides used. In all cases the antiseptic action (that is, antiseptic action due to incomplete washing) of the disinfectant was prevented only when it hindered normal germination and growth of the seed. The percentage of germination and appearance of growth were the indicators in each experiment.

The first germicide employed in this work was commercial chloride of lime (largely calcium hypochlorite), as suggested by Wilson ('15), and his method was followed, namely, "Ten grams of commercial chloride of lime (titrating 28 per cent chlorine) is mixed with 140 cc. of water. The mixture is then allowed to settle for five or ten minutes and the supernatant liquid decanted off or filtered. The solution or filtrate, which contains about 2 per cent chlorine, is used as the disinfectant. The volume of the solution employed should be about five times or more the volume of the seed."

The seeds were placed in glass tumblers, in quantity not to exceed about one-fifth of the final volume. The tumblers were

then filled with the above-mentioned filtrate and covered with the top of a sterile Petri dish. The seeds were then removed at intervals of 2, 6, 11, and 20 hours. This was done with sterile forceps in the case of large seed and by means of a platinum spoon with small seed. In this case it was only necessary to drain the seed of the hypochlorite solution, as the antiseptic action did not hinder the germination and development of the seed. The transfer was made to small flasks or Petri dishes (depending on the size of the seed). In these flasks and Petri dishes there had been previously placed a small amount of a synthetic nutrient medium[1] in 1.5 per cent agar. Usually from 10 to 20 seeds were placed in each vessel. The flasks and Petri dishes were then placed in an incubator maintained at 25° C. The varieties of seed used in this experiment are given in table I. Not many kinds could be tried, but it will be noted that those experimented upon represent seeds of widely different families. The seeds were obtained from the Vaughan Seed Company, Chicago, Illinois, in all cases except where note is made to the contrary. More uniformity in results was obtained when seeds were secured from the same company. The results of the disinfectant are shown in table I.

According to the results indicated in the table, the commercial "chloride of lime" was very effective for sugar beet, cucumber, and lettuce, a higher percentage of germination and more vigorous seedlings being obtained by the use of this disinfectant. The most effective time intervals are shown in table IV. However, many seeds of other plants were obtained free from contamination, but the effectiveness of the germicide was not sufficiently high for practical purposes. Certain cucumber seed obtained from other seed companies could not be sterilized even with this disinfectant. Bacteria would continue to grow at the micropylar end of such of these seed as

[1] The nutrient medium was made up as follows:

Ammonium nitrate	1 gm.
Dihydrogen potassium phosphate	.5 gm.
Magnesium sulphate	.25 gm.
Iron chloride	Trace
Cane sugar	5 gms.
Distilled water	500 cc.

TABLE I

TREATMENT OF SEEDS WITH CALCIUM HYPOCHLORITE SOLUTION

Seed	Time of treatment (hrs.)	Germination (per cent)	Contamination after 48 hrs. (per cent)	Condition of seedling
Cucumber (*Cucumis sativus*)	2	95	20	Good
	6	95	15	Good
	11	95	0	Very good
	20	95	0	Injured
Bean (*Phaseolus vulgaris* var. *nanus*)	2	45	20	Fair
	6	16	0	Poor
	11	16	0	Poor
	20	10	0	Poor
Radish (*Raphanus sativus*)	2	80	50	Growth inhibited
	6	24	40	
	11	20	40	
	20	20	30	
Lettuce (*Lactuca sativa*)	2	96	0	Good
	6	96	0	Good
	11	50	0	Poor
	20	0	0	Poor
Sunflower (*Helianthus annuus*)	2	90	100	Mouldy
	6	90	100	
	11	90	100	
	20	60	100	Poor
Sugar beet (*Beta vulgaris*)	2	40	10	Normal
	6	40	0	Normal
	11	40	0	
	20	25	0	Injured
Cotton (*Gossypium*)*	6	85	100	Normal but seed covered with Fusarium
	11	60	100	
	20	60	100	
	44	0	100	
Corn (*Zea Mays*)†	2	90	60	Good
	6	90	20	Good
	11	80	10	Good
	20	20	0	Poor
Alfalfa (*Medicago sativa*)	2	80	40	Good
	6	80	20	Good
	11	80	5	Good
	20	40	0	Growth inhibited
Turnip (*Brassica Rapa*)	2	80	40	Good
	6	80	10	Fair
	11	25	5	Growth inhibited
	20	0	0	Growth inhibited
Vetch (*Vicia sativa*)	2	90	20	Fair
	6	90	10	Growth inhibited
	11	40	0	
	20	0	0	
Cucumber (*Cucumis sativus*)†	2	60	60	Normal
	6	60	30	
	11	40	10	
	20	20	5	

* Seed from Alabama. † Seed from St. Louis Seed Co.

remained viable after 20 hours' treatment with the hypochlorite solution. This, however, either indicates that disinfection did not occur or that the bacteria were shielded by being so far within as to be beyond the range of diffusion of the disinfectant. This disinfectant, then, is only effective with cer-

tain seeds, but by extensive tests many more might be added to the list.

Inasmuch as it is not possible to include a test which is in every sense a control, it may be well to state at this point that the germination of all seed was tested out under usual conditions for germination and in every case ranged from 95 to 100 per cent.

The second disinfectant employed was formaldehyde. It is especially toxic to many parasitic fungi inducing plant diseases. In practically all cases where it has been unsuccessfully used as a general germicide, the failure has been due rather to its injurious action upon the seed than to ineffectiveness in controlling fungi and bacteria. Seed injury has been especially noticeable where the gas method has been used. If the seed contains a large amount of water, the gas is absorbed more readily and the seed is promptly injured.

The method employed was the same as the previous one except that the seeds had to be washed free of the disinfectant. It was found after many trials that one thorough rinsing in sterile water was sufficient to remove enough of the formaldehyde to prevent the antiseptic action from affecting the germination and development of the seed. The time intervals for removing seed from the solution were usually 15, 30, and 45 minutes. The effectiveness of the fungicide is shown in table II. Formaldehyde was very effective for a large proportion of the seed used. In most cases, better results were obtained when the seeds were left in a stronger solution a shorter time. Excellent results were obtained when the seeds were first dipped momentarily in 70 per cent alcohol, or for a definite preliminary period in hydrogen peroxide. Where the seeds are fairly resistant this method of treatment is the logical one. Only fair results could be obtained with either cotton or potatoes. In the case of the former, the seed had to be delinted with concentrated H_2SO_4 and then subjected to the formaldehyde treatment. In the case of the potato it was difficult to prevent bacterial growth. Eyes with a very small portion of the potato (1 c. cm.) were washed for twelve hours,

TABLE II
TREATMENT OF SEEDS WITH FORMALDEHYDE

Seed	Time of treatment (minutes)	Germination (per cent)	Contamination (per cent)	Condition of seedling
2 per cent solution				
Dwarf bean	15	96	20	Normal
	30	80	20	Normal
	45	80	10	Normal
Cucumber	15	92	15	Normal
	30	80	5	Fair
	45	60	0	Poor
Lettuce	15	96	50	Fair
	30	50	0	Poor
	45	10	0	Very poor
Radish	15	90	5	Good
	30	45	0	Fair
	45	30	0	Very poor
	60	4	0	Very poor
Sugar beet	15	40	5	Very poor
	30	0	0	Very poor
	45	0	0	Very poor
Sunflower	15	85	50	Normal
	30	66	15	Normal
	45	55	15	Normal
Wheat*	15	90	5	Very good
	30	96	0	Very good
	45	92	0	Very good
Barley	15	80	10	Fair
	30	20	5	Poor
	45	0	0	
Corn	15	90	20	Good
	30	90	10	Good
	45	60	0	Fair
Garden pea	15	90	20	Normal
	30	80	0	Normal
	45	40	0	Injured
3 per cent solution				
Dwarf bean	15	96	40	Normal
	30	98	0	Good
	45	40	0	Fair
Cucumber	15	90	10	Normal
	30	96	0	Normal
	45	96	0	Poor
Cotton	15	60	80	Poor
	30	40	80	Poor
	45	40	70	Poor
Cotton¶	15	60	80	Normal
	30	60	80	Normal
	45	50	5	Normal
	60	40	0	Injured
Sunflower	15	90	80	Fair
	30	96	70	Fair
	45	70	40	Poor
Sunflower**	15	96	5	Good
	30	96	0	Good
	45	70	0	Growth inhibited
Wheat††	15	96	15	Good
	30	96	0	Good
	45	90	0	Fair
Alfalfa	15	90	20	Normal
	30	90	0	Normal
	45	60	0	Injured
Corn	15	90	0	Normal
	30	90	0	Slightly injured
	45	40	0	Injured
Potato	15	100	90	Normal
	30	100	90	Normal
	45	90	80	Normal
	60	90	80	Normal

* Preliminary treatment—soaked in water 12 hrs.
** Preliminary treatment—soaked in water 18 hrs.
¶ Preliminary treatment—soaked in water 18 hrs., heated to 68° C. for 15 min.
†† Preliminary treatment—soaked in water 6 hrs.

TABLE II (continued)

Seed	Time of treatment (minutes)	Germination (per cent)	Contamination (per cent)	Condition of seedling	Seed	Time of treatment (minutes)	Germination (per cent)	Contamination (per cent)	Condition of seedling
4 per cent solution									
Cotton	15	60	80	Normal	Potato	15	100	90	Normal
	30	60	60	Normal		30	100	60	Normal
	45	40	20	Slightly injured		45	90	20	Normal
	60	20	20	Injured		60	40	10	Injured
Cotton†	15	60	40	Normal	Potato‡‡	15	100	90	Normal
	30	60	10	Normal		30	100	40	Normal
	45	40	0	Slightly injured		45	80	10	Normal
	60	20	0	Injured		60	40	10	Injured
Cotton‡	15	60	40	Normal	Potato‖‖	15	100	40	Normal
	30	50	10	Normal		30	100	10	Normal
	45	40	0	Slightly injured		45	60	0	Normal
	60	0	0	Injured		60	20	0	Slightly injured
Cotton‖	15	60	10	Normal	Sunflower	15	20	0	Injured
	30	50	0	Normal		30	0	0	.....
	45	40	0	Slightly injured		45	0	0	.....
	60	0	0	Injured		60	0	0	.....
Potato 5% HCHO	15	100	50	Normal	Bean	15	90	0	Normal
	30	80	40	Normal		30	90	0	Injured
	45	60	20	Injured		45	20	0	Badly injured
	60	20	10	Injured		60	0	0	.....

† Preliminary treatment—soaked in 1% H_2O_2 for 10 min.
‡ Preliminary treatment—soaked in water 18 hrs., then heated to 65° C. for 15 min.
‖ Lint removed with concentrated H_2SO_4.
‡‡ Preliminary treatment—cubes treated with 70% alcohol for 1 min.
‖‖ Preliminary treatment (see text).

then dipped momentarily in 70 per cent alcohol, then into a 1 per cent H_2O_2 solution for 10 minutes, and finally into the 4 or 5 per cent formaldehyde for 45 minutes. The percentage of germination was low, but after sprouts appeared growth was normal. In a few cases, sterile plants could not be obtained, and this was related to the source and kind of potatoes.

Alcohol was the next disinfectant tried. The same method as the foregoing was employed. The seeds were washed once to prevent further injurious action. No table of results is here included, as I was unable to free any seed from bacteria and still obtain fair germination. The same kinds of seeds as were used in table II were employed in this experiment. Weak alcoholic solutions (10–25 per cent) were used for long periods of time and stronger (25–70 per cent) for short periods as the seed would allow. In only a few cases were sterile seed obtained. Alcohol cannot be entirely discarded as a disinfectant, as it is very effective in a subsidiary way, as when seeds are first immersed in strong alcohol and then followed by another disinfectant. It serves to remove air cavities which would not otherwise allow proper contact for the disinfectant.

The next disinfectant used was mercuric chloride. This has also been used very extensively for seed treatment. It is effective in dilute solutions, and for this reason it is necessary to remove practically all the solution from the seed, as its antiseptic action prevents the germination and growth of the seed. Some seeds are very easily injured by dilutions that will not kill certain fungi or bacteria. This is especially true with barley. It is also effectively used as the chief disinfectant when preceded by hydrogen peroxide or alcohol. The method used was the same as given with the previous disinfectants. The seeds were washed thoroughly of the $HgCl_2$, as its antiseptic action was found to be injurious in most cases. I was able to disinfect many seeds with mercuric chloride that could not be freed from contamination by any other disinfectant. Table III shows the results with this disinfectant.

It might be stated again that the seeds used in this table could not in every case be made free from contamination, but when good viable seed were used, a high percentage of the trials proved successful. Many hundred plantlets were obtained from seed disinfected by the above methods. From the data at hand it appears that a previous treatment with a good bactericide, such as alcohol or hydrogen peroxide, is to be desired. There are many seeds that will withstand a suf-

TABLE III

TREATMENT OF SEEDS WITH MERCURIC CHLORIDE

Seed	Time of exposure (min.)	Germination (per cent)	Contamination (per cent)	Condition of seedling
.1 per cent solution				
Corn	1	90	40	Normal
	10	90	20	Normal
	20	80	0	Normal
	40	40	0	Injured
Field peas	1	96	30	Normal
	10	96	10	Normal
	20	96	0	Normal
	40	60	0	Injured
Wheat	1	90	35	Normal
	10	10	10	Normal
	20	20	5	Injured
	40	0	0	Injured
Dwarf bean	1	96	60	Normal
	10	96	40	Normal
	20	60	10	Injured
	40	20	5	Injured
Radish	1	90	60	Normal
	10	90	50	Normal
	20	90	20	Normal
	40	60	0	Normal
Alfalfa	1	86	70	Normal
	10	78	60	Normal
	20	70	10	Normal
	40	60	0	Normal
Garden peas	1	96	75	Normal
	10	96	70	Normal
	20	96	40	Normal
	40	90	20	Normal
Barley	1	60	75	Normal
	10	60	5	Normal
	20	40	0	Injured
	40	0	0	
.5 per cent solution				
Garden peas	1	96	40	Normal
	10	96	20	Normal
	20	90	0	Normal
	40	80	0	Slightly injured
Dwarf bean	1	94	40	Normal
	10	94	10	Normal
	20	70	0	Normal
	40	30	0	Injured
Garden cress (*Lepidium sativum*)	1	90	40	Normal
	10	90	20	Normal
	20	80	0	Normal
	40	30	0	Injured
Morning glory (*Ipomoea purpurea*)	1	96	20	Normal
	10	96	10	Normal
	20	90	0	Normal
	40	60	0	Injured
Nasturtium (dwarf)	1	90	60	Normal
	10	90	40	Normal
	20	90	0	Normal
	40	90	0	Injured
Onion*	1	80	90	Normal
	10	80	40	Normal
	20	80	0	Normal
	40	60	0	Injured
Lupinus albus†	1	90	80	Normal
	10	90	80	Normal
	20	90	60	Normal
	40	80	10	Normal
Lupinus albus‡	1	90	60	Normal
	10	90	50	Normal
	20	90	10	Normal
	40	75	0	Normal

* Preliminary treatment—seed soaked in H_2O 12 hrs., then 1% H_2O_2 for 1 min.
† Preliminary treatment—soaked in 70% alcohol for 1 min.
‡ Preliminary treatment—soaked in H_2O_2 for 10 min.

ficiently long treatment of both agents mentioned, and some, such as barley, that are very sensitive and cannot be so treated. I have not been able to free barley from contamination in more than 75 per cent of the seed used. There are many other seeds that are easily killed by any germicide that will be effective against the adhering microörganisms. In conclusion, therefore, it would seem necessary to continue to treat the various seed differently and with different disinfectants. One of the most important considerations is that there are undoubtedly different contaminating organisms.

TABLE IV

SUMMARY GIVING BEST TREATMENTS FOR VARIOUS KINDS OF SEEDS

(For percentage of germination in each case, see Tables I, II, and III)

Disinfectant	Seed	Strength of disinfectant	Time of exposure	Previous treatment
Calcium hypochlorite ($CaOCl_2$)	Cucumber	Full	11 hrs.	None
	Lettuce	Full	2–6 hrs.	None
	Sugar beet	Full	11 hrs.	70% alcohol, 1 min.
Formaldehyde (HCHO)	Bean	2–3%	30 min.	None
	Cotton	3–4%	45 min.	Soaked in H_2O, 12 hrs., lint removed with H_2SO_4
	Corn	2–3%	15 min.	None
	Cucumber	2–3%	30 min.	None
	Alfalfa	3%	30 min.	None
	Sunflower	2–3%	30 min.	Soaked in H_2O, 18 hrs.
	Wheat	2–3%	30 min.	Soaked in H_2O, 6 hrs., 1% H_2O_2, 10 min.
	Potato	3–4%	45 min.	70% alcohol, 1 min.
Mercuric chloride ($HgCl_2$)	Corn	.1%	20 min.	None
	Field peas	.1%	20 min.	None
	Radish	.1%	40 min.	None
	Alfalfa	.1%	40 min.	None
	Barley	.1%	10 min.	70% alcohol momentarily
	Garden peas	.1%	20 min.	None
	Dwarf bean	.5%	20 min.	None
	Garden cress	.5%	20 min.	None
	Nasturtium	.5%	20 min.	Soaked in H_2O, 12 hrs.
	Onion	.5%	20 min.	Soaked in H_2O, 12 hrs., then in 1% H_2O_2, 10 min.
	Lupines	.5%	40 min.	1% H_2O_2, 10 min.; 70% alcohol, 1 min.

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SEED DISINFECTION FOR PURE CULTURE WORK: THE USE OF HYPOCHLORITES

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INTRODUCTORY

For a long time it has been clear that much light may be thrown on many fundamental problems in seed plant nutrition as well as in physiological pathology through the use of pure cultures of certain seed plants. In those cases where the seed are produced in pods, solid fruits, or within other thoroughly protective coverings, it is, as a rule, a relatively simple matter to secure seed in season entirely free from contamination. It is only necessary to employ the usual bacteriological precautions, opening the maturing pods or fruits with care and removing the seed to sterile containers, in which they may be kept until required. Beans, peas, radishes, tobacco, tomatoes, and various cucurbits or melons are among those plants easily handled in this way. The difficulty, however, even with these seed, lies in anticipating what may be needed out of season. With the majority of seed, moreover, it would not be practicable to use the isolation method either because of structural difficulties or of inaccessibility of fruiting plants.

The experiments of Wilson[1] on the use of commercial chloride of lime (in part calcium hypochlorite) have been the first definite application of the value of this well-known disinfecting agent to plant physiological study with seed plants. In the disinfection and antiseptic treatment of wounds, extensive studies have been made during this war with the use of hypochlorous acid, the hypochlorites, and related compounds, as a result especially of the investigations of Dakin[2]

[1] Wilson, J. K. Calcium hypochlorite as a seed sterilizer. Am. Jour. Bot. 2: 420–427. 1915.

[2] Dakin, H. D., and E. K. Dunham. A handbook on antiseptics. 126 pp. New York, 1917.

and his associates. In general, this work has emphasized the value of a series of compounds containing "active chlorine," by which term Dakin infers a connotation of "the ability of any particular substance to part with chlorine, free or combined, in such a way that it can effect the chlorination of bacterial and other proteins." Through the NH groups of their constituent amino-acids the proteins are subject to attack by such chlorinated agents, whereby in the first step the Cl is substituted for the H-atom in the group mentioned with the formation of chloramines.

Materials and Methods

In the preliminary work here reported we have not departed from the readily obtainable commercial products, namely, (1) commercial chloride of lime, or "bleaching powder," (2) "chlorinated potassa" (a liquid product recognized by certain St. Louis manufacturers as "eau de Javel"), (3) solid sodium hypochlorite, and (4) Dakin's soluble chloramine T. These we have compared with a few standard disinfectants of other groups. The commercial products vary somewhat in composition, but these differences, in our experience, are not so great as to interfere with this type of practical work. A careful study of standardized preparations is, however, planned. Chloramine T, or chlorazene, is the abbreviated or trade name for sodium-toluene-para-sulphochloramide. No experimental work was done with the other Dakin products of this class.

The chlorinated lime, designated 10 per cent, was prepared in the following way: Ten grams of a standard commercial product were stirred into 100 cc. distilled water. After standing 10 minutes the supernatant liquid was filtered and the filtrate employed. Other concentrations were prepared in an analogous manner. The commercial Javel water was used as if it were a pure substance, 10 and 20 per cent solutions referring respectively to the use of 10 and 20 cc. of the commercial product with enough water in each case to make 100 cc. All necessary precautions have been taken to prevent accidental contamination. The technique of handling treated seed

has been invariably carried out in a transfer room repeatedly steamed to insure the precipitation and fixation of dust particles. In some cases the seed were preliminarily immersed in running water for from 4 to 16 hours, in other cases dry seed were treated directly.

In the earlier work the selected seed were placed in small cheese-cloth bags, and these immersed in covered vessels containing the disinfecting solutions employed. After the interval of treatment the bags were transferred to jars of sterile distilled water for from 15 minutes to 1 hour, and when it seemed desirable a second quick rinsing was given. The contents of each bag was then carefully dumped into a sterile Petri dish. In later work the washed or soaked seed were carefully placed in sterile Erlenmeyer flasks, and the disinfectant then poured in, a separate flask being used for each lot of seed to be treated for any interval of time as well as by each concentration of disinfecting solution. After treatment the seed were twice shaken with sterile water, the second wash water remaining not less than 15 minutes. The seed were then transferred to a Petri dish by means of a metal spoon. All implements employed were sterilized by dipping in alcohol and then promptly burning this off.

The purity and germination of the seed were then followed after their transfer to large Petri dishes containing standard potato decoction agar. Care was taken to insure intimate contact of the seed with the medium. From 15 to 50 seed, depending upon size, were usually arranged in each dish, and each test duplicated.

Inasmuch as the essential thing in such work is to obtain a high percentage of germinating seed free from contamination and readily transferable to other cultures, the above methods have seemed entirely adequate, and complicated apparatus, such as that devised by de Zeeuw[1], is not merely unnecessary, but it is in general impracticable.

In the true sense of the word "control" experiments are not possible in this work, and no attempt is made to include ex-

[1] Zeeuw, R. de. The comparative viability of seeds, fungi and bacteria when subjected to various chemical agents. Centralbl. f. Bakt. II. 31 : 4–23. *1 f.* 1911.

periments thus designated. All seed not disinfected will exhibit contamination on agar. On the other hand, elaborate experiments might have been made to determine the effect of the disinfectant upon the seed, and in a certain sense these would serve as controls. Our object, however, has been simply to use seed which under the usual conditions of the germinator exhibit a high percentage of germination, so we have merely assured ourselves of the capacity of the seed employed to germinate satisfactorily. It is admitted that germination in a Petri dish on agar is not comparable to germination in a germinator.

Experimental Data and Discussion

At the outset it should be definitely acknowledged that as a general principle the practically perfect disinfection of seed by chemical agents is only possible when the contaminating organisms of the seed are superficial, or largely superficial. This conclusion is drawn from a variety of observations and experiments, the general result of which is too obvious to require elaborate data and discussion but merits mention in respect to some pronounced instances. Experiments have been made with seed suspected of more than superficial contamination in the case of corn, sunflower, squash, tomato, and *Melilotus*. In the first case, corn of the 1917 crop was obtained in which a discoloration of the micropylar end of the seed was characteristic. The normal maturity of this crop in the Middle West was more or less affected by early frost, and in some sections subsequent wet weather induced visible mouldiness. The seed used exhibited no macroscopically visible infection of the cob, but such infection was inferred. After treatment with various disinfecting agents the majority of these seed and any contaminating organisms were either killed by the agent, or, in the agar cultures, there was a growth of fungous hyphae from the discolored micropylar end. Such seed were necessarily discarded. The other seed mentioned had been stored under moist conditions, favoring the development of moulds and bacteria, and in no case was it possible to disinfect any reasonable percentage without injury in respect to

the capacity for vigorous germination. Similar observations upon the seed of certain grasses, sorghums, etc., which had undergone considerable heating during the curing process have led to the conviction that penetration of the seed by microörganisms, especially fungi, is not infrequent, and therefore a certain "purity" of the seed employed is requisite.

In table I are shown data obtained from the treatment of Canada field peas and corn with chlorinated lime, chlorinated potash, and chlorazene. In this case the first-mentioned disinfectant was more injurious to peas than in any other test made. The results with chlorinated potash were considered particularly good for the preliminary trials, while the results with chlorazene were disappointing.

Before proceeding further, tests were made, for comparison, with formalin and mercuric bichloride, and a second trial of chlorazene was included, as given in table II. The use of mercuric bichloride with such seed gave perfect disinfection, but these, as well as subsequent experiments, seemed to indicate that whenever this result was accomplished the injury to the seed was considerable. Various grades of alcohol, from 20 to 95 per cent, were also used in this series, the treatment

TABLE I

DISINFECTION OF SEED (AFTER IMMERSION IN WATER FOR 16 HOURS) BY CHLORINATED LIME, CHLORINATED POTASH (JAVEL WATER), AND CHLORAZENE. FINAL OBSERVATIONS AFTER 72 HOURS, ROOM TEMPERATURE

	Disinfectant	Treatment 3½ hours		Treatment ½ hour	
		% Germination	% Contamination	% Germination	% Contamination
Peas	25% chlor. lime	12	0	25	0
	15% chlor. lime	6	0	...	....
	25% chlor. potash	88	0	100	0
	10% chlor. potash	94	0	88	0
	4% chlorazene	0	4	0	0
	2% chlorazene	6	3–4	25	0
	1% chlorazene	42	0	87	0
Corn	25% chlor. lime	80	0	87	0
	15% chlor. lime	75	7	80	7
	25% chlor. potash	88	0	100	3
	10% chlor. potash	94	3–4	100	0
	4% chlorazene	94	0	100	11
	2% chlorazene	88	7	66	10
	1% chlorazene	75	0	87	7

TABLE II

DISINFECTION OF CANADA FIELD PEAS BY FORMALIN, MERCURIC BICHLORIDE, AND CHLORAZENE. FINAL OBSERVATIONS AFTER 96 HOURS, 26° C.

Disinfectant	Treatment 2 hours			Treatment 1 hour		
	% Germination	% Contamination	Condition	% Germination	% Contamination	Condition
* .2% formalin	77	50	Fair	93	10	Good
.2% formalin	70	66	Poor	83	43	Good
* .1% $HgCl_2$	16	0	Injured	24	0	Injured
.1% $HgCl_2$	47	0	Fair	76	0	Fair
*4% chlorazene	0	10	Injured	0	50	Fair
4% chlorazene	16	40	Fair	56	13	Fair
*2% chlorazene	33	6	Fair	43	6	Good
2% chlorazene	40	33	Good	63	6	Good
*1% chlorazene	86	20	Good	80	10	Good
1% chlorazene	83	30	Good	80	30	Good

* In these cases the seed were soaked for 16 hours prior to treatment, while in the other cases dry seed were treated.

being 5, 20, and 60 minutes. After 60 hours practically every seed was contaminated, so that the results are not tabulated.

In table III are given further results with chlorinated lime and potash. The intervals employed are too short for best results, so that the percentage of contamination runs high. Moreover, these experiments were made several months later than those included in tables I–II, and the seed were not so fresh. It seems clear, however, that short treatments are not satisfactory, and the value of longer intervals is particularly emphasized later.

A further extensive test of chlorinated lime, employing intervals of treatment up to 3 hours and using the freshest seed available, was made with the special view of determining the effect of the agent on the germination of the seed. The results are given in detail in table IV. The effectiveness of this disinfectant with corn and cucumber is clear. The percentage of germination with peas and radish is relatively low. This lot of radish seed proved difficult to sterilize, and the presence of a resistant organism spreading rapidly over the surface of the dishes tended to reduce the percentage of germination.

The test of sodium hypochlorite was made by preparing the concentrations indicated in table V from the solid substance.

TABLE III

DISINFECTION OF SEED BY CHLORINATED LIME AND CHLORINATED POTASH. FINAL OBSERVATIONS AFTER 96 HOURS

	Disinfectant	Treatment 1 hour			Treatment ½ hour		
		% Germination	% Contamination	Condition	% Germination	% Contamination	Condition
Peas	*20% chlor. lime	34	3	Fair	23	17	Fair
	20% chlor. lime	100	6	Good	87	0	Good
	*10% chlor. lime	37	13	Good	77	3	Fair
	10% chlor. lime	93	13	Good	93	0	Good
	* 5% chlor. lime	75	6	Fair	77	General	Fair
	5% chlor. lime	75	0	Good	100	0	Good
	*20% chlor. potash	80	20	.....	80	6	.....
	20% chlor. potash	75	13	.....	87	6	Good
	*10% chlor. potash	77	22	Good	77	50	Good
	10% chlor. potash	87	6	Good	93	6	Good
	* 5% chlor. potash	94	16	Good	77	50	Good
	5% chlor. potash	93	13	Good	87	0	Good
Corn	*20% chlor. lime	83	0	Fair	89	0	Good
	20% chlor. lime	88	20	Good	67	0	Good
	*10% chlor. lime	97	6	Good	67	0	Fair
	10% chlor. lime	95	6	Good	72	0	Fair
	* 5% chlor. lime	88	6	Good	78	10	Fair
	5% chlor. lime	88	0	Good	88	0	Fair
	*20% chlor. potash	75	3	Good	75	0	.....
	20% chlor. potash	67	6	Good	77	0	.....
	*10% chlor. potash	86	0	Good	67	General	Fair
	10% chlor. potash	83	13	Good	83	0	Fair
	* 5% chlor. potash	81	23	Good	75	General	Fair
	5% chlor. potash	83	6	Good	77	General	Fair

* In these cases the seed were soaked for four hours prior to treatment, while in the other cases dry seed were treated.

Although rather more erratic than results with other hypochlorites, the trial was of importance inasmuch as some dishes were free, or practically free, of contamination, and the percentage of germination relatively high. After further tests, however, it appeared that stronger solutions might be employed, and later a series of experiments was made in which this agent was compared with the potassium salt and with chlorazene, as shown by the data in table VI. These results are much more favorable than before for sodium hypochlorite; but the uniformly high percentage of germination, the good condition of the seedlings, and the freedom from contamina-

TABLE IV

DISINFECTION OF SEED (AFTER IMMERSION IN WATER FOR 4 HOURS) BY CHLORINATED LIME. FINAL OBSERVATIONS AFTER 96 HOURS

Kind of seed	% Concentration	Interval of treatment, hours	% Germination	% Contamination
Corn	10	2	72	0
	10	1	90	0
	10	½	90	0
	5	3	72	2
	5	2	84	0
	5	1	86	6
Peas	10	2	12	0
	10	1	24	0
	10	½	18	4
	5	3	38	0
	5	2	24	6
	5	1	32	4
Cucumber	10	2	84	0
	10	1	84	0
	10	½	68	2
	5	3	64	0
	5	2	82	8
	5	1	84	88
Radish	10	2	12	18
	10	1	30	14
	10	½	38	14
	5	3	4	56
	5	2	8	58
	5	1	28	24

tion (not perfect in the case of radish) where the potassium salt was employed, mark this as far more reliable under these conditions.

During the progress of this work other investigations were in progress by one of us and by graduate students, in which germinating seed free of contaminating organisms were required. The Javel treatment was employed, and with the consent of those who coöperated in this work, Dr. W. W. Bonns and Mr. T. Matsumoto, we are enabled to report the following facts: Of 500 seed of Canada field peas treated 3 hours with a 10 per cent solution the germination was 90 per cent and the contamination less than 1 per cent. In treating tobacco seed, 2- and 3-hour intervals proved inadequate; but a 4-hour interval with 12 per cent Javel water gave a contamination of less

TABLE V

DISINFECTION OF SEED (AFTER IMMERSION IN WATER FOR 4 HOURS AT 23°C.) BY SODIUM HYPOCHLORITE. FINAL OBSERVATIONS AFTER 96 HOURS

Kind of seed	% Concentration	Interval of treatment, hours	% Germination	% Contamination
Corn	10	2	96	2
	10	1	86	30
	10	½	92	4
	5	3	90	0
	5	2	92	0
	5	1	90	44
Peas	10	2	26	35
	10	1	30	96
	10	½	58	52
	5	3	40	58
	5	2	28	30
	5	1	30	20
Cucumber	10	2	88	50
	10	1	96	72
	10	½	92	100
	5	3	92	30
	5	2	82	14
	5	1	94	98
Radish	10	2	26	58
	10	1	18	10
	10	½	46	14
	5	3	46	6
	5	2	24	2
	5	1	26	4

than 2 per cent when 12 plates of about 30 seed each were sown. At the same time small quantities (2 dishes each) of lettuce, navy beans, and lima beans were similarly treated, with no contamination in any dish.

It was suggested that discontinuous disinfection might prove serviceable and practicable in work of this type, just as discontinuous sterilization by heat is so effective. Accordingly, experiments were arranged in which the dry seed were treated for 2 hours in deep Petri dishes with 20 per cent Javel water, then, after pouring this off, the seed were rinsed, and finally left in an incubator at room temperature for 2 days. The same treatment with rinsing was then repeated, and the seed placed for germination as in other cases. The results are shown in table VII, and these indicate complete disinfec-

TABLE VI

DISINFECTION OF SEED (AFTER IMMERSION IN WATER FOR 4 HOURS AT 23° C.) BY CHLORAZENE, CHLORINATED POTASH, AND SODIUM HYPOCHLORITE. FINAL OBSERVATIONS AFTER 96 HOURS

Disinfectant	Kind of seed	% Germination	% Contamination	Condition
1% chlorazene	Corn	100	50	Good
	Peas	26	100	Good
	Cucumber	100	...	Injured
	Radish	40	100	Good
20% chlor. potash	Corn	96	0	Good
	Peas	94	0	Good
	Cucumber	84	0	Good
	Radish	64	16	Good
20% sodium-hypochlorite	Corn	100	2	Good
	Peas	22	50	Injured
	Cucumber	96	0	Good
	Radish	12	0	Good

tion, as well as an unusually high percentage germination as compared with the usual percentage when the same seed are treated with any such disinfecting agent and placed directly on agar. The principle involved may possibly be widely applicable and deserves consideration in other work.

The results of similar tests with chlorinated lime, varying, however, the time intervals and the concentration of the disinfectant, are given in table VIII.

In addition to the results reported in full, extensive experiments were made with sodium chlorate and calcium sulphite. Concentrations of 10 and 5 per cent of the pure reagents were employed for various intervals, but in all cases 100 per cent

TABLE VII

DISINFECTION OF SEED BY 20 PER CENT CHLORINATED POTASH,—DISCONTINUOUS TREATMENT. FINAL OBSERVATIONS AFTER 96 HOURS

Kind of seed	% Germination	% Contamination	Condition
Corn	100	0	Fine
Peas	90	0	Good
Cucumber	100	0	Fine
Radish	50	20	Fair

TABLE VIII

DISINFECTION OF SEED BY CHLORINATED LIME,—DISCONTINUOUS TREATMENT. FINAL OBSERVATIONS AFTER 96 HOURS

Kind of seed	% Concentration	Interval of treatment, hours	% Germination	% Contamination
Corn	10	1	72	0
	*10	1	60	0
	*10	½	100	0
	5	2	56	28
	*5	2	100	0
	*5	1	96	0
Peas	10	1	20	8
	*10	1	52	0
	*10	½	24	50
	5	2	16	100
	*5	2	60	0
	*5	1	40	4
Cucumber	10	1	92	0
	*10	1	36	0
	*10	½	80	0
	5	2	60	100
	*5	2	72	12
	*5	1	100	0
Radish	10	1	0	12
	*10	1	8	16
	*10	½	14	100
	5	2	0	48
	*5	2	0	56
	*5	1	18	...

* In these cases the seed were soaked for 4 hours prior to treatment, while in the other cases dry seed were treated.

of contamination occurred, so that these and related substances were believed to be unsatisfactory for the purpose of this study.

Particular stress has been laid by de Zeeuw on the difficulty of properly washing off the disinfectant. He argues that quite commonly there may be transferred with the seed a sufficient amount of the disinfectant to insure antiseptic action during the germination of the seed on agar, but the unkilled germs may develop later—when the seed are transferred to final cultures. With the hypochlorites we have not been able to detect any such possibility, even when intentionally making the washing process less thorough than usual. Seed of beans,

corn, and peas, merely dipped in water—after disinfection—and then once in a dilute suspension of *Bacillus mycoides* before being placed on agar for germination, showed 100 per cent of contamination after repeated trials. Possible difficulties with the salts of heavy metals were not tested.

Definite experiments are under way to test the value of certain commercial hypochlorites in the disinfection of seed for agricultural purposes.

Summary Recommendation

As a practical result of the experiments on seed treatment reported it is believed that, taking into consideration both (1) the capacity of the seed for germination after treatment and (2) the relative freedom from contamination by micro-örganisms, the commercial "chlorinated potassa," or Javel water, is the most satisfactory agent which has yet been considered. The length of time required for practically perfect disinfection of certain seed in our experiments, with concentrations of 10–20 per cent, is 3 hours or longer. In some cases interrupted disinfection fully warrants the extra trouble and delay. The value of soaking the seed previous to the longer interval treatments is doubtful. Nevertheless, a preliminary thorough washing of all seed, with removal of decayed and imperfect ones, is advisable. A study of standardized hypochlorites is essential.